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SUPERCONSERVED RECEPTORS EXPRESSED IN THE BRAIN EXPRESSION, FUNCTION, MOTIFS AND EVOLUTION OF AN ORPHAN RECEPTOR FAMILY

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ABSTRACT

GPR27, GPR85 and GPR173 constitute a small family of G protein-coupled receptors (GPCR) that share the distinctive characteristics of being highly conserved throughout vertebrate evolution and predominantly expressed in the brain. Accordingly, they have been coined as "Superconserved Receptors Expressed in the Brain" (SREB), although their expression profile is more complex than what was originally thought. SREBs have no known validated endogenous ligands and are thus labeled as "orphan" receptors. The investigation of this particular category of uncharacterized receptors holds great promise both in terms of physiology and drug development. In the largest GPCR family, the Rhodopsin-like or Class A, around 100 receptors are considered orphans. Because GPCRs are the most successful source of drug targets, the discovery of a novel function or ligand most likely will lead to significant breakthroughs for the discovery of innovative therapies.

The high level of conservation is one of the characteristic features of the SREBs. We propose herein a detailed analysis of the putative evolutionary origin of this family. We highlight the properties that distinguish SREBs from other rhodopsin-like GPCRs. We present the current evidence for these receptors downstream signaling pathways and functions. We discuss the pharmacological challenge for the identification of natural or synthetic ligands of orphan receptors like SREBs. The different SREB-related scientific questions are presented with a highlight on what should be addressed in the near future, including the confirmation of published evidence and their validation as drug targets. In particular, we discuss in which pathological conditions these receptors may be of great relevance to solve unmet medical needs.

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1. Introduction

G protein-coupled receptors (GPCR) are seven transmembrane helices proteins that are essential to transduce a wide range of signals to the cell. They constitute the largest family of proteins, with 850 members encoded in ~2% of the human genome (Lander et al., 2001). They are generally divided between "physiological" (~365 members in humans) and "sensory" receptors (~435 members in humans for olfaction, vision, taste and pheromones) (Alexander et al., 2021; Bjarnadóttir et al., 2006). Several classifications for GPCR families have been proposed but for the present work, we will use the "GRAFS" system that relies on strict phylogenetic criteria (Fredriksson, Lagerström, Lundin, & Schiöth, 2003). GPCRs are involved in nearly all physiological processes and are the direct target of ~35% of the currently marketed drug collection (Hauser, Attwood, Rask-Andersen, Schiöth, & Gloriam, 2017; Sriram & Insel, 2018). Despite this obvious therapeutic potential and the immense research effort on GPCRs, the family remains largely understudied. The *International Union of Basic and* Clinical Pharmacology (IUPHAR) estimates that around 100 receptors are still orphans, which means they lack a validated endogenous ligand (Davenport et al., 2013; Laschet, Dupuis, & Hanson, 2018). A close look at the recent literature shows that most orphan GPCRs are poorly investigated (Laschet et al., 2018; Roth & Kroeze, 2015). This is due in part to the limited availability of pharmacological tools (such as ligands) to study them (Edwards et al., 2011; Roth & Kroeze, 2015).

Most orphan GPCRs are not grouped in clusters but GPR27, GPR85 and GPR173 constitute a small subfamily called the "Super Conserved Receptors expressed in the Brain" (SREB) in the rhodopsin-like subgroup of GPCRs. Their name reflects two of their attributes: i) expression in the central nervous system and ii) a surprisingly high degree of conservation throughout vertebrate evolution (see below) (Matsumoto et al., 2000). The SREBs were initially classified in the prostaglandin receptor cluster by Fredriksson et al. (Fredriksson et al., 2003) although they exhibit amino acid sequence homology with amine receptors (see Table 1) (Matsumoto et al., 2000; O'Dowd et al., 1998).

Table 1. Homologies in percent amino acid identity between the human SREBs with representative receptors of the human aminergic family. (source: UNIprot).

	GPR27	**GPR85**	**GPR173**
GPR27	100%	51.3%	52.1%
GPR85	51.3%	100%	62.3%
GPR173	52.1%	62.3%	100%
$ADRB_2$	15.5%	18.1%	17.8%
D_2	16.8%	16.1%	16.9%
$5HT_{1A}$	17.3%	16.8%	19.5%
M_2	16.6%	16.5%	17.5%
H_1	13.0%	14.9%	15.2%

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The first member of the SREBs to be identified was the human GPR27 and its mouse ortholog. Their coding sequences were independently discovered by two different labs using primarily Expressed Sequence Tags (EST) database screening, based on the dopamine receptor D_4 sequence (Matsumoto et al., 2000; O'Dowd et al., 1998). Matsumoto et al. proposed to group GPR27 (or SREB1) with two other receptors exhibiting a high degree of homology, SREB2 (GPR85) and SREB3 (GPR173), discovered in an EST database using the GPR27 template, to form the SREB GPCR subfamily (Matsumoto et al., 2000; O'Dowd et al., 1998). At the same time, a second group independently reported the presence of human and mouse GPR85 in the brain and its resemblance to GPR27 (Hellebrand, Chica Schaller, & Wittenberger, 2000).

In humans, GPR27 is an intronless gene, a common feature of vertebrate rhodopsin-like GPCRs (Strotmann et al., 2011), located on chromosome 3, whereas GPR173 and GPR85 have multiple exons with a coding sequence not interrupted by an intron. They are located on chromosome X and 7, respectively (Matsumoto et al., 2000). In the GRAFS system, they are classified in the α group (more precisely the prostaglandin receptor cluster) of the rhodopsin-like family (called "class A" in other classifications) (Fredriksson et al., 2003). However, besides their inclusion in the rhodopsin-like (or class A) receptors, a definitive classification inside this family is not straightforward as they have been either defined as an isolated cluster (Kakarala & Jamil, 2014) or clustered with purinergic (Civelli et al., 2013) or amine (Matsumoto et al., 2000) receptors. At the protein level, SREBs share the highest sequence similarity (around 15–20%) with aminergic GPCRs (Table 1).

2. Evolutionary aspects

The high level of conservation is one of the characteristic features of the SREBs. Therefore, we propose a detailed analysis of the putative evolutionary origin of this family. GPCRs are considered as ancient proteins, being present in all eukaryotes (Schöneberg, Hofreiter, Schulz, & Römpler, 2007), including yeast (Elion, 2000), insects (Hill et al., 2002) and plants (Tuteja, 2009). The typical architecture of seven transmembrane helices can also be found in bacteria (Soppa, 1994) although these may have arisen from convergent evolution rather than from a shared common origin, given their low sequence similarity.

The Chordata phylum includes organisms that lack an internal cartilaginous or bony skeleton (protochordates; namely tunicates and lancelets) or have an internal skeleton (subphylum Vertebrata). Database analyses of the available genomes for invertebrates of the urochordata lineage (*Ciona intestinalis* & Ciona savignyi) revealed no receptor with close homology to any of the SREB members. In three lancelets (Branchiostoma floridae, *Branchiostoma belcheri, Asymmetron lucayanum*), we found a protein similar to SREBs (Table 2). Lancelets diverged from other chordate lineages (urochordate and vertebrate) about 550 million years ago (Chen, 2008; Delsuc, Brinkmann, Chourrout, & Philippe, 2006; Huang et al., 2014; Whittaker, 1997). This implies that the first SREB (GPR85 or SREB2, due to the highest amino acid identity to human GPR85, Table 2) probably originated at that time. The lancelet SREB receptor homolog likely originated from the duplication of a common ancestor of the aminergic family, for which it is known that the serotonin receptors

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family is among the evolutionary oldest in the rhodopsin-like receptors family (Schöneberg et al., 2007).

Table 2. Homology as % amino acid identity between human SREB and receptor homologs identified in lancelets, lamprey and hagfish as well as cartilaginous fishes (Accession numbers are listed in Table S1). The corresponding alignment is shown in Supplementary Fig. S2.

	Human GPR85	**Human GPR173**	**Human GPR27**
Lancelets			
SREB / GPR85	39%	37%	33%
Lampreys & hagfish			
GPR85–1	57%	56%	47%
GPR85–2	56%	57%	44%
GPR173	58%	60%	50%
Cartilaginous fishes			
GPR85	79%	63%	49%
GPR27	64%	70%	52%

Vertebrates are further divided into those organisms that lack a hinged jaw (*Agnatha*, hagfishes or lampreys) and those organisms that have a hinged jaw (Gnathostoma, including the cartilaginous fishes, bony fishes, amphibians, reptiles, birds, and mammals) (Dores, 2011). Our database mining revealed evidence for three genes orthologous to SREBs in lampreys (Petromyzon marinus, *Lethenteron reissneri*, *Lethenteron camtschaticum*, *Entosphenus tridentatus*) and one hagfish (*Eptatretus burger*) for which phylogenetic analyses showed that two of them grouped with other GPR85 orthologs, while the last was found in the branch of GPR173 (Supplementary Figs. S1, S2). This suggests that the origin of the two SREB paralogs GPR85 and GPR173 can be dated back to the last common ancestor of lampreys and hagfishes.

In the cartilaginous fishes (Chondrichthyes) including *Callorhinchus milii* (elephant shark), *Rhincodon typus* (whale shark) and *Amblyraja radiata* (thorny skate), we detected genes for GPR85 and GPR27. In the very diverse clade of ray-finned fishes (*Actinopterygii*), most species have all three SREB paralogs (Fig. 1, Supplementary Table S1). In coelacanth (Latimeria *chalumnae*), one of the earliest bony fish, all three SREBs can be found, while in *Neoceratodus forsteri* (Australian lungfish), we only found evidence for the presence of GPR85 and GPR173 (Fig. 1, Supplementary Table S1). A recent study provided detailed analyses of SREB evolution in ray-finned fish highlighting differences in genomic location, absence and presence of SREB genes in different orders, which therefore will not be discussed in detail here (Breton et al., 2021). All amphibians exhibit both GPR85 and GPR173, but our database analyses revealed that GPR27 was only present in frogs and toads (Anura). In contrast, we found that all sauropsids possess GPR85 and GPR27, while GPR173 is only present in

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Lepidosauria and absent in all birds and crocodiles (Fig. 1, Supplementary Fig. S3 and Supplementary Table S1). In the mammalian genomes, we found GPR85 and GPR173, while GPR27 was absent or only partially present in some species throughout all mammalian orders without a recognizable pattern (Fig. 1, Supplementary Fig. S4, Supplementary Tables S1 and S2).

The exceptional conservation of the SREBs has been highlighted as one of their landmark features since their identification (Hellebrand et al., 2000; Matsumoto et al., 2000). These seminal observations regarding SREB conservation have never been updated in the light of the very extensive genome sequencing of the last few years. Our database interrogations revealed that GPR85 is present and highly conserved (greater than 99% amino acid identity across 186 species, Supplementary Table S3) in all vertebrates and its origin dates back as far as the lancelets, belonging to the protochordates (Fig. 1). Similarly, it is known that among GPCRs, frizzled receptors (FZD), which play a crucial role in eumetazoan ontogenesis including regulation of cell proliferation, cell polarity, gastrulation and tissue formation, exhibit highest conservation in vertebrates and even in invertebrates (Strotmann et al., 2011). Further, rhodopsin (RHO), melanocortin receptor 4 (MC4R) and β_2-adrenergic receptor (β_2AR) are considered to be highly conserved throughout evolution (Devic, Xiang, Gould, & Kobilka, 2001; Stäubert et al., 2007). To highlight the extraordinary evolutionary conservation of SREBs, we analyzed the amino acid identity of GPR85 and compared it to FZD4, RHO and MC4R (Supplementary Table S3). In this comparison, we included 186 species for which sequence data for all four GPCRs was publicly available and found GPR85 to be the most conserved (Table 3 and Supplementary Table S3). Analyses of a smaller set (92 species, Supplementary Table S4) of orthologs of GPCRs of the SREB family compared to highly conserved proteins, including one histone (H3A3) and one heat shock protein (HSPA5), GPR85 was found less conserved than H3A3 but more than HSPA5 (Table 3). GPR173 exhibits higher sequence identity across 92 species than FZD7, RHO, MC4R and FZD5, while GPR27 is the least conserved but still shows a higher sequence identity than β_2AR (Table 3). This striking evolutionary conservation of SREBs indicates that they are under strong negative (purifying) selection, i.e. randomly occurring (deleterious) mutations were removed during evolution. This is especially true for GPR85, while our analyses indicate that GPR27 and GPR173 might have redundant functions since several species throughout all orders lack one of these two SREB orthologs. One would expect that such a strong degree of conservation indicates that the respective protein is crucial for the survival of most species. However, so far the generated SREB knock-out mice for GPR85 (Matsumoto et al., 2008) or GPR27 (Chopra, Yiv, Hennings, Zhang, & Ku, 2020) are viable and have mild phenotypes (see detailed information in section 4 below).

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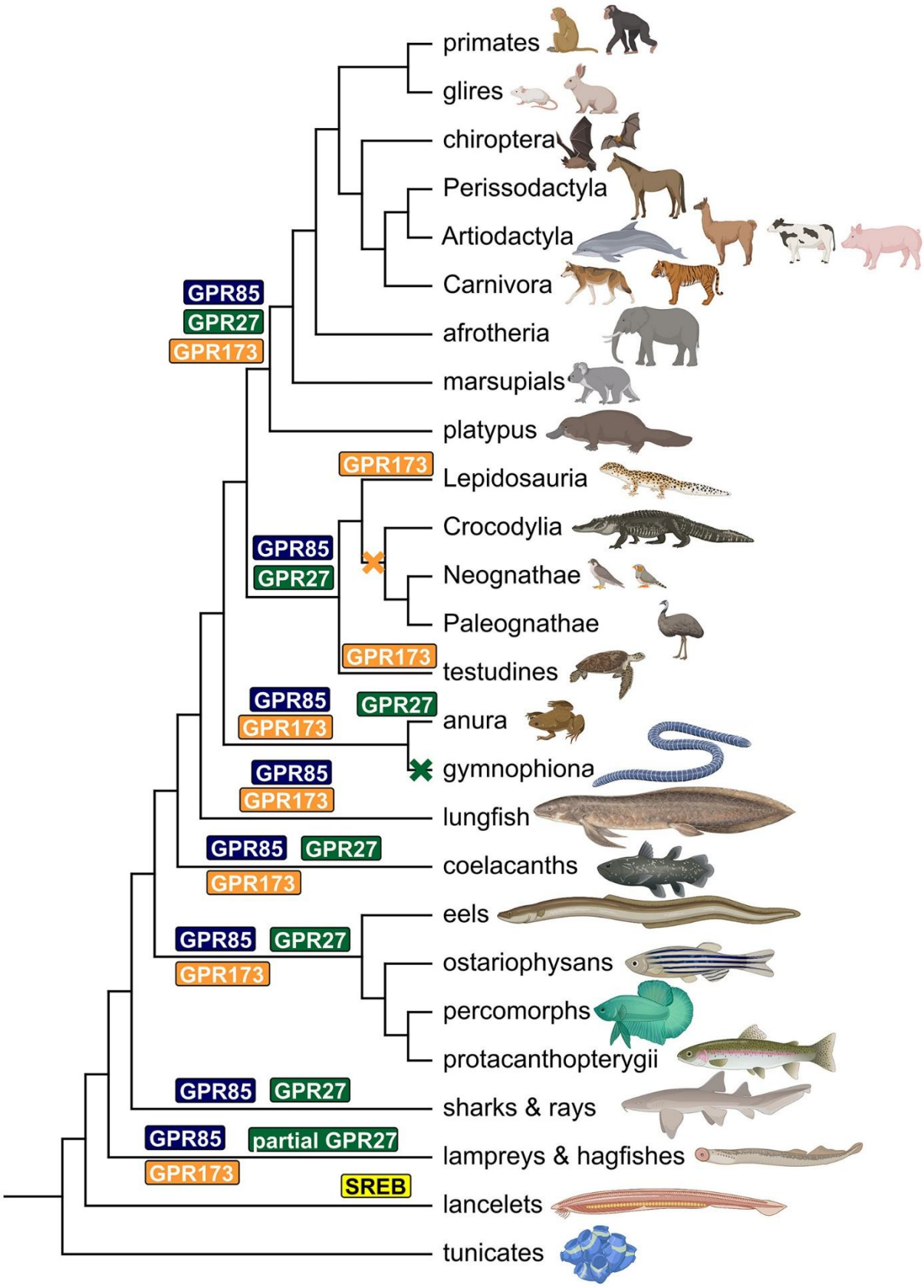

Fig. 1. Schematic phylogenetic tree of SREBs indicating their origins and losses during vertebrate evolution.

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Table 3. Conservation among species. NCBI Accession numbers for all species and orthologs included in the analyses are listed in Tables S3 and S4.

Protein	Number of orthologs	% amino acid identity (mean ± SD)
GPR85	186	99.3 ± 0.5
FZD3	186	94.5 ± 4.7
MC4R	186	89.6 ± 3.2
RHO	186	89.2 ± 3.2
H3A3	92	100
GPR85	92	99.2 ± 0.7
HSPA5	92	97.2 ± 1.6
FZD3	92	96.7 ± 1.9
GPR173	92	94.8 ± 4.6
FZD7	92	92.9 ± 4.5
RHO	92	91.9 ± 3.6
MC4R	92	91.3 ± 3.9
FZD5	92	90.7 ± 6.4
GPR27	92	87.9 ± 9.1
ADRB2	92	82.1 ± 6.5

3. Molecular and structural aspects

GPCRs can be seen as complex allosteric machines characterized by conformational rearrangement upon ligand binding/activation that enables the transduction of the signal across the cell membrane. The different residues linking ligand binding to receptor activation and intracellular transducer binding, so-called "microswitches", are evolutionarily conserved (Filipek, 2019). Consequently, although they display a wide variety of structures, it is generally accepted that, inside the same family of receptors such as the Rhodopsin-like GPCRs, the activation mechanism is similar and eventually leads to coordinated movement of transmembrane helices, notably the rotation and displacement of helix 6 and helix 5 that create a space to accommodate the binding of transducer molecules (Smith, 2021; Venkatakrishnan et al., 2016; Zhou et al., 2019). To gain some insight into the molecular and structural aspects of the SREBs, we have established the snake plots of GPR85, GPR27 and GPR173 (Fig. 2, left) based on the transmembrane helices (TM) predictions made by the server TMHMM (https://services.healthtech.dtu.dk/service.php?TMHMM-2.0). This simulation

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slightly differs from the ones obtained with other similar tools (such as the one provided by "GPCRdb" (Isberg et al., 2016; Kooistra et al., 2021)). In addition, we added the Alphafold (Jumper et al., 2021; Varadi et al., 2022) predictions for the three SREBs (Fig. 2, right). In the absence of resolved 3D structures of these receptors, it is not possible to verify which one is the closest to the real situation. However, these models are useful to catch some notable characteristics of this subfamily of orphan receptors, and the different elements discussed below are based on the snake plots in Fig. 2. Interestingly, some of the typical motifs involved in receptor activation significantly diverge in the SREBs compared to other Rhodopsin-like GPCRs. We will briefly discuss some of these presumably important atypical SREB motifs and structural elements as they may guide future investigations regarding their functions and ligands.

3.1. CONNECTING LOOPS

In their overall architecture, the three SREB receptors display the typical arrangement of rhodopsin-like GPCRs. They have a relatively short N- and C-terminus. However, the three receptors share an unusually long ECL2 and ICL3 (Fig. 2). These elements may play significant roles for the receptors' function and/or mechanism of activation.

3.1.1. ECL2

Initially thought to have no specific function besides their contribution to the overall structure, the extracellular loops of GPCRs, the ECL2 in particular, have now been demonstrated to fulfill an important role in several receptors (Szpakowska, Perez Bercoff, & Chevigné, 2014; Wheatley et al., 2012; Woolley & Conner, 2017). The ECL2 can be disordered but also take the shape of ordered β-sheets (e.g. rhodopsin, CXCR4, S1P1) or α-helices (e.g. βARs, A_{2A}) (Nicoli, Dunkel, Giorgino, de Graaf, & Di Pizio, 2022). As such, the ECL2 constitutes a lid partially or completely covering the binding site (Hanson et al., 2012; Nygaard, Frimurer, Holst, Rosenkilde, & Schwartz, 2009), contributes to ligand binding kinetics (Grundmann et al., 2016; Szpakowska et al., 2018; Wang et al., 2018; Smith et al., 2011) and selectivity (Magnani et al., 2014; Woolley & Conner, 2017), regulates surface expression (Hoffmann, Moro, Nicholas, Harden, & Jacobson, 1999) or directly activates the receptor (Lin et al., 2020). In addition, the ECL2, which is usually the longest extracellular loop, is characterized by a highly conserved disulfide bond with the cysteine located at the top of TM3 ($Cys^{3.25}$) that imposes conformational constraints to the receptor (Pal & Chattopadhyay, 2019; Wheatley et al., 2012). ECL2 can also form additional disulfide bridges, notably with the N-terminus (GPR39) (Storjohann, Holst, & Schwartz, 2008) or ECL1 (A_{2a}) (Jaakola et al., 2008). These various structures and conformations are important for a diverse array of functions depending on the nature of the receptor. For instance, in GPR39, the disulfide bridge with the N-terminus negatively influences receptor activity (Storjohann et al., 2008). GPR52, an orphan rhodopsin-like GPCR unrelated to the SREBs, is another relevant example for the importance of ECL2. In this case, the loop binds within the orthosteric pocket of GPR52 and switches the receptor in an active state (Lin et al., 2020). As for the SREBs, their long ECL2 and the presence of predicted sites for disulfide bridges (Fig. 2) suggest that the loop is also playing a key function. For example, it is conceivable that ECL2 places the receptor into an inactive state as the SREBs seem to be devoid of significant basal activity (Dupuis et al., 2017).

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Targeted mutagenesis aiming at the disruption of ECL2 or the determination of the SREBs inactive and active state structure would bring useful information to answer these questions.

3.1.2. ICL3

The ICL3 is another important loop for GPCR function. Recent studies revealed that ICL3 may adopt transient secondary structures during activation that contribute to the receptor function (Du et al., 2019). SREBs share a relatively large ICL3 (Fig. 2) that is well conserved among them but differs markedly from other GPCRs. The role of the ICL3 has been substantially investigated in other rhodopsin-like receptors. This intracellular loop has been shown to modulate the interaction of receptors with various transducers and intracellular effectors such as G proteins (e.g. the dopamine D_2 receptor (Żuk, Bartuzi, Matosiuk, & Kaczor, 2020)), arrestins (e.g. the H_4 receptor (Verweij et al., 2020)), Src family kinases (e.g. the β_3-Adrenergic receptor, β_3AR, reviewed in (Berndt & Liebscher, 2021)), or G protein-coupled receptors kinases (GRK) (Verweij et al., 2020). Regarding the SREBs, our prediction analyses using MoDPepInt (Kundu, Mann, Costa, & Backofen, 2014) revealed several Src-homology 3 (SH3) protein-binding motifs in SREBs ICL3 (Table S5). The importance of these motifs has been highlighted in other receptors. For example, the D_4 receptor has an SH3 binding motif in its ICL3 that interacts with the adaptor proteins Grb2 and Nck. The disruption of these interactions results in impaired signaling such as the loss of adenylyl cyclase modulation or $ERK_{1/2}$ phosphorylation, although the binding of G proteins is not affected (reviewed in (Magalhaes, Dunn, & Ferguson, 2012)). Thus, the identification of SH3 domain-containing proteins that interact with ICL3 of SREBs may shed light on signal transduction pathways activated by SREBs independently of heterotrimeric G proteins.

3.2. TRANSMEMBRANE HELICES AND CONSERVED MOTIFS

The molecular mechanisms leading to receptor activation are incompletely understood. The collection of structural and mutagenesis data has only started to give a more precise insight into this complex process. Undoubtedly, the numerous conserved motifs and microswitches buried inside the receptors play a critical role in their activation (reviewed in (Hauser et al., 2021)). Intriguingly, most of these conserved elements are not present or differ significantly in the SREBs. In addition, the data collected so far have failed to convincingly link the SREB activation to G protein-mediated pathways (see below). This atypical behavior might well take its roots in the SREBs' peculiar motifs.

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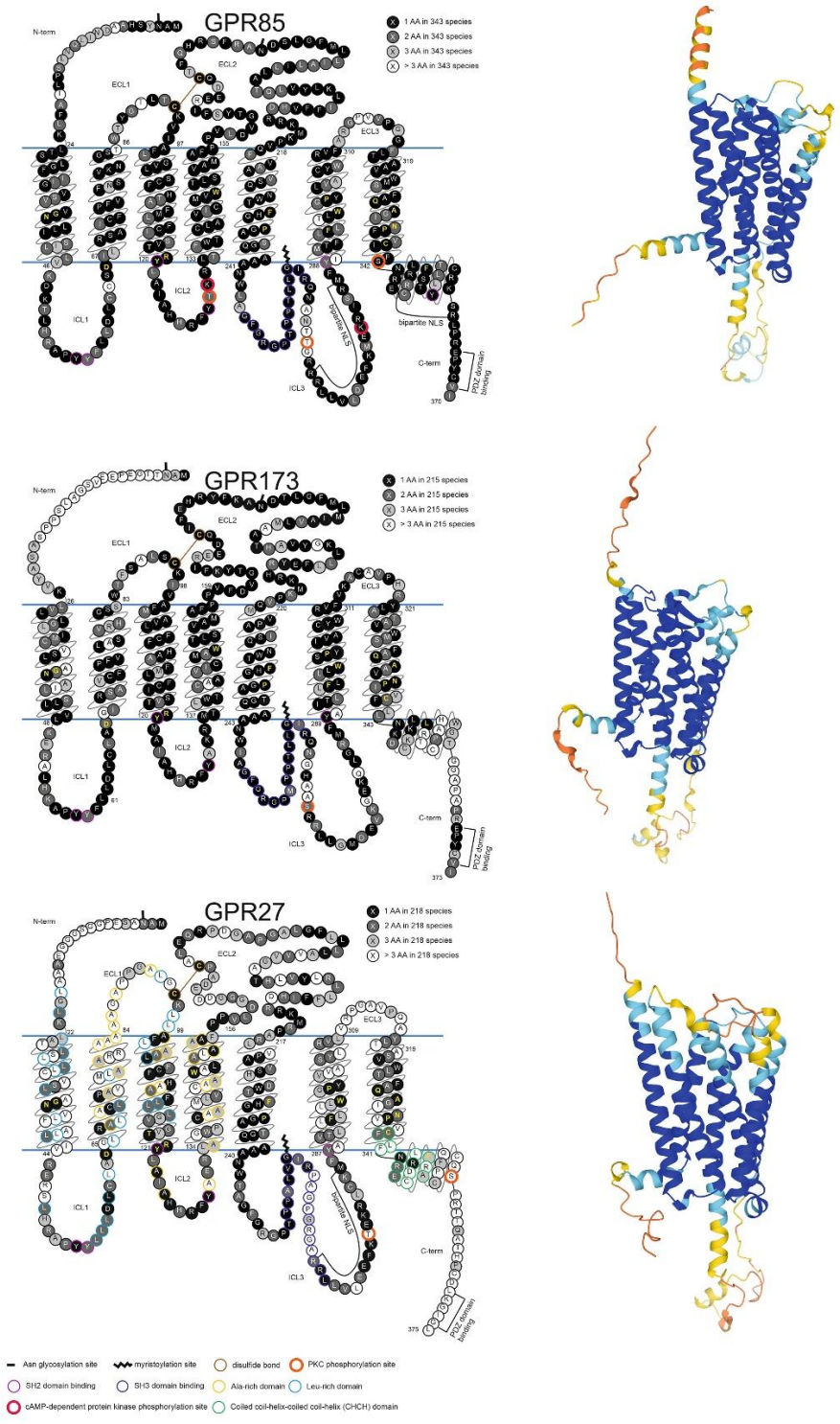

Fig. 2. Structure prediction for the three SREB receptors. Left: Snake plots presenting the amino acid sequence, obtained at Uniprot (https://www.uniprot.org), of the receptors with the transmembrane helices (TM) predictions made by the server TMHMM (http://www.cbs.dtu.dk/services/TMHMM/). Motif Scan (https://myhits.sib.swiss/cgi-bin/motif_scan) was used to find all known motifs (Table S6) that occur in SREBs (Prosite on ExPASy, Pfam and InterPro). Right: tertiary structure prediction of each receptor provided by Alphafold (https://alphafold.ebi.ac.uk). Alphafold/uniport entries: P60893 (Human GPR85), Q9NS67 (Human GPR27) and Q9NS66 (Human GPR173).

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3.2.1. SODIUM BINDING POCKET

The existence of a negative allosteric effect of sodium (Na^+) on agonist potencies has long been recognized for several receptors (Katritch et al., 2014; Pert, Pasternak, & Snyder, 1973; Strasser, Wittmann, Schneider, & Seifert, 2015; Zarzycka, Zaidi, Roth, & Katritch, 2019; Zou et al., 2021). The high resolution structure of the A_{2A} adenosine receptor was the first that unambiguously identified a Na^+ ion bound to the receptor in a deep allosteric pocket (Liu et al., 2012). Subsequent structural determinations of GPCRs in different functional states confirmed the existence of a conserved Na^+ binding pocket in several receptors (Zarzycka et al., 2019) (Table 4). Current models propose that, upon activation of the receptor, the Na^+ binding pocket collapses, rendering the binding/presence of Na^+ and agonists mutually exclusive (Katritch et al., 2014). The ligand can also directly take the place of the sodium ion (Filipek, 2019). The Na^+ pocket, although not identical in all receptors crystallized so far, is, in its minimal form, composed of four conserved amino acids (Katritch et al., 2014), namely $D^{2.50}$, $S^{3.39}$, $N^{7.45}$ and $N^{7.49}$ (Superscript indicates Ballesteros-Weinstein numbering system (Ballesteros & Weinstein, 1995)). The establishment of a salt bridge between Na^+ and the highly conserved $D^{2.50}$ seems to be particularly important. The regulation of the degree of constitutive activity can also be influenced by the stabilizing presence of Na^+. Thus, the absence of Na^+ in external medium or buffers can confer, in receptors having a Na^+-binding pocket, a higher level of constitutive activity. This is illustrated for example by the D_4 receptor (Wang et al., 2017).

Table 4. Main residues involved in the stabilization of Na^+ in GPCR structures (Katritch et al., 2014). The residues displayed as "cons" are the most commonly seen in Rhodopsin-like receptors (source: GPCRdb (Isberg et al., 2016)).

Na^+ Pocket	**1.50**	**1.53**	**2.46**	**2.47**	**2.49**	**2.50**	**3.35**	**3.39**	**3.43**	**6.44**	**6.48**	**7.45**	**7.46**	**7.49**	**7.50**	**7.53**
CONS	N	V	L	A	A	D	N	S	L	F	W	N	S	N	P	Y
GPR27	N	F	L	C	A	D	L	L	L	F	W	Q	A	N	P	C
GPR85	N	I	L	C	S	D	S	T	L	F	W	Q	A	N	P	C
GPR173	N	L	L	C	A	D	F	A	L	F	W	Q	A	N	P	C
β_2AR	N	V	L	A	A	D	L	S	L	F	W	N	S	N	P	Y

Although some of the residues of the typical Na^+-binding pocket differ in the SREBs compared to other closely related receptors (Table 4), they have not been listed as receptors presumably lacking such a Na^+ binding pocket (Katritch et al., 2014). Thus, the generation of mutants affecting the putative Na^+ binding pocket of SREBs or experiments measuring basal activity with varying concentrations of Na^+ may provide a useful tool to generate constitutively active receptors and facilitate the investigation of SREBs coupling and downstream signaling pathway.

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3.2.2. CWXP AND PIF

The *CWxP* motif is located inside helix 6 at the bottom of the ligand-binding pocket. The central $W^{6.48}$ is usually referred to as the "tryptophan rotamer toggle switch" (Holst et al., 2010; Zhou et al., 2019). This name derives from the fact that the indole side chain of the $W^{6.48}$ undergoes rotameric conformational changes between receptors in complex with agonists or antagonists (Eddy et al., 2018). The temporary rotameric movement of the residue leads to a movement of water molecules and displacement of other residues that eventually provokes the alteration of the transmembrane helix bundle (Filipek, 2019; Zhou et al., 2019). This central tryptophan toggle switch ($W^{6.48}$) is conserved in the SREBs, but the cysteine $C^{6.47}$ is replaced by a leucine (L) residue whereas the $P^{6.50}$ is present. This C → L replacement points towards a possible atypical activation mode or $W^{6.48}$ placement in the SREBs.

The $W^{6.48}$ rotamer toggle switch functions in a larger "transmission switch" in the helix 3–5-6 interface that involves neighboring residues, notably the "PIF" motif (Deupi & Standfuss, 2011; Filipek, 2019; Zhou et al., 2019). This conserved triad motif in the receptor core consists of $P^{5.50}$ – $I^{3.40}$ – $F^{6.44}$ and conveys important rearrangements during GPCR activation (Huang et al., 2015; Ishchenko et al., 2017; Kato et al., 2019; Rasmussen et al., 2011). The PIF form is the most common but some receptors have an alanine at the 3.40 position and thus a "PAF" motif instead, such as in the neurotensin receptor 1(Kato et al., 2019). The SREB family has such a PAF triad, a variation that presumably does not affect its function. According to our models (Fig. 2), the $F^{6.44}$ and $P^{5.50}$ are also conserved in the SREB.

3.2.3. DRY

The (E/D)RY motif is located at the end of TM3 and the beginning of the ICL2 (Rovati, Capra, & Neubig, 2007). It is one of the most important microswitches involved in GPCR activation as it is thought to contribute to the stabilization of the receptor's inactive state (Zhou et al., 2019). The first residue is a D or an E in ~86% of the Rhodopsin-like family (Mirzadegan, Benkö, Filipek, & Palczewski, 2003). An aromatic amino acid is found in 82% of GPCR at the 3.51 position, the Y being the most common (67% of all receptors, replaced by F or W in 11% and 4% of the receptors, respectively) (Lu, Curtis, Jones, Pavia, & Hulme, 1997; Mirzadegan et al., 2003; Wess, 1998). The central arginine is the most conserved residue in rhodopsin-like GPCRs, with only ~4% of receptors bearing another residue, often resulting in altered functionality (Rosenkilde, Kledal, & Schwartz, 2005). The motif is important for receptor activation (Zhou et al., 2019) but also, together with ICL2, for optimal interaction with G proteins or arrestins (Carpenter & Tate, 2017; Flanagan, 2005; Marion, Oakley, Kim, Caron, & Barak, 2006). Initially, the existence of an "ionic lock", i.e. a salt bridge between two highly conserved basic and acidic residues was postulated to exist between $R^{3.50}$ and the neighboring $D/E^{3.49}$ and/or more distant charged residues such as $D/E^{6.30}$ on helix 6 (Ballesteros et al., 2001; Rovati et al., 2007). The existence of a salt bridge between helices 3 and 6 has been validated in several inactive state structures (Filipek, 2019; Rosenbaum, Rasmussen, & Kobilka, 2009) but it seems to be more common between $R^{3.50}$ and $D/E^{3.49}$ (Trzaskowski et al., 2012). The disruption of an ionic lock involving the residues of the DRY motif is a mainstay of the current paradigm describing the rhodopsin-like GPCRs activation mechanism (Zhou et al., 2019). Furthermore, the release of $R^{3.50}$ from an ionic lock is a

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critical step to accommodate transducer binding as $R^{3.50}$ is interacting in several active state complexes with the C-terminal helix of the Gα protein and with the finger loop of arrestin (Carpenter & Tate, 2017).

Interestingly, the DRY motif in TM3 is not present in SREBs but is conserved as a "TRY" motif (Table 5). According to our search of the databases, this alternative version of the motif is found only in the Relaxin family peptide receptor 3 (RXFP3) and GPR141 (source GPCRdb). The "TRY" motif is anticipated to be important for some specific aspects of SREB function, notably the process of their molecular activation (presence of an ionic lock in the inactive receptor) or their interaction with transducers (Rovati et al., 2007).

Table 5. The "DRY" motif and ICL2 of various GPCRs.

DRY-ICL2	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
CONS	D	R	Y	L	A	I	V	H	P	L	R	Y	R	R	L	R	T	P	R	R
							T	Y				S		T					T	
GPR27	T	R	Y	L	A	I	A	H	H	R	F	Y	A	E	R	L	A	G	W	P
GPR85	T	R	Y	L	A	I	A	H	H	R	F	Y	T	K	R	L	T	F	W	T
GPR173	T	R	Y	M	A	I	A	H	H	R	F	Y	A	K	R	M	T	L	W	T
B1AR	D	R	Y	L	A	I	T	S	P	F	R	Y	Q	S	L	L	T	R	A	R
B2AR	D	R	Y	F	A	I	T	S	P	F	K	Y	Q	S	L	L	T	K	N	K
B3AR	D	R	Y	L	A	V	T	N	P	L	R	Y	G	A	L	V	T	K	R	C
Rl3r1	T	R	Y	H	S	V	A	S	A	L	K	S	H	R	T	R	G	H	G	R
GnRHR	D	R	S	L	A	I	T	R	P	L	A	L	K	S	N	S	K	V	G	Q
V2	D	R	H	R	A	I	C	R	P	M	L	A	Y	R	H	G	S	G	A	H

3.2.4. NPXXY

This motif, located at the end of helix 7, is a major microswitch involved in the conformational changes associated with GPCR activation (Rosenbaum et al., 2009). The diverse structural determinations of receptors have shown that the highly conserved proline 7.50 of the motif induced a distortion in helix 7. The $Y^{7.53}$ functions as a permanent toggle switch at the late stages of the conformational transition from inactive to active state (Filipek, 2019; Venkatakrishnan et al., 2016; Zhou et al., 2019). Furthermore, this residue is one of the three (with $Y^{5.58}$ and $R^{3.50}$) that undergoes similar conformational movement in all established active receptor-transducer complexes (Carpenter & Tate, 2017; Wang, Hua, & Liu, 2020). Interestingly, although it is conserved in most Rhodopsin-like receptors, it is not present in the SREBs, where the motif has the form *NPxxC*. The

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modification NPxxY$^{7.53}$ → NPxxC$^{7.53}$ is a unique feature among rhodopsin-like GPCRs. Interestingly, the other highly conserved rotamer toggle switch Y$^{5.58}$ is also absent in SREBs.

The fact that several key typical GPCR structural features are not present in SREBs may point towards different possibilities regarding their function: 1. The SREBs are atypical GPCRs that function differently and lack classical G protein-mediated signaling and/or arrestin binding; 2. They have non-canonical molecular architecture and active conformations that diverge from the typical activation scheme; 3. They have protein interaction partners different from those known for most rhodopsin-like GPCRs; 4. They do not have endogenous ligands but are activated through interaction or loss of interaction with their structural extracellular features, in a fashion similar to the adhesion GPCRs (Paavola & Hall, 2012); 5. They have an internal ligand encoded within the protein sequence of SREBs; 6. SREBs or fragments of SREBs have intracellular functions interacting with proteins of different compartments mediating cell polarization, differentiation or division. The iProt-Sub database (https://iprot-sub.erc.monash.edu/) enables analyses of protein sequences for predicted protease cleavage sites and several such conserved sites were identified in SREBs (Table S7) potentially hinting towards functions independent of G protein- or arrestin-coupling. For GPR50, which is also an orphan GPCR, usage of an alternative signal transduction mode relying on the nuclear translocation of its C-terminal domain after cleavage by calpain protease, has recently been reported (Ahmad et al., 2020). Thus, alternative modes of activation and/or signaling are conceivable for SREBs.

4. Expression, function and putative endogenous ligands

In this section, we provide a brief overview of the current knowledge regarding the expression and function of the SREBs. In accordance with their names, they are predominantly expressed in the central nervous system (Matsumoto et al., 2000; Regard, Sato, & Coughlin, 2008). Interestingly, early reports suggested their presence also in peripheral tissues (Matsumoto et al., 2000; Regard et al., 2008). Some of the available data indicate that SREBs can be involved in the regulation of the excitatory postsynaptic core scaffolding protein "SH3 And Multiple Ankyrin Repeat Domains 3" (SHANK3) gene expression and provoke molecular changes in different regions of the brain, such as medial prefrontal cortex (mPFC) or/and striatum. For example, in a transcriptome analysis of transgenic mice overexpressing Shank3, the mRNA level of gpr85 was found to be increased in multiple brain regions, while the mRNA levels of gpr27 and gpr173 were decreased in the cortex and striatum. These observations were also confirmed in neuronal cell lines, which further highlight that SREBs may play a crucial role in the synaptic development and its function modulation (Jin et al., 2018). Recently, several genes coding for the three receptors were found to be expressed in fish gonads, which indicates, according to the authors, that the function of the SREBs may differ, both in genomic structure and their functions (Breton et al., 2021).

So far, few reports have directly addressed the question of the function of this family of receptors. Several endogenous ligands have been proposed for the SREBs, but so far none of them has been validated (confirmed by at least two independent labs) according to the IUPHAR recommendations

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(Davenport et al., 2013; Laschet et al., 2018). Identification of the endogenous SREB ligands and their physiological functions may indeed reveal insights into previously unsuspected fundamental neuronal systems of vertebrates.

We discuss below for each receptor a summary of their putative role reported in the literature.

4.1.1. GPR27

The expression of GPR27 (SREB1) has been analyzed at the mRNA level, in human tissues (Amisten, Salehi, Rorsman, Jones, & Persaud, 2013; Matsumoto et al., 2000), in mouse tissues (Regard et al., 2008) and in brains from Rhesus macaque monkeys (Matsumoto et al., 2005). While detectable throughout the whole brain, the highest levels of mRNA expression were found in the striatum (caudate nucleus, putamen), the hippocampus (notably the dentate gyrus), subthalamic nuclei, olfactory bulb, pituitary gland and supraoptic nucleus of the hypothalamus. Lower levels were also detected in the cerebellum (Matsumoto et al., 2000; Regard et al., 2008). At the peripheral level, GPR27 mRNA is present in ovary, testis, heart (ventricule), prostate, pancreas (islets of Langerhans), and at lower levels in small intestine, urinary bladder, uterus and peripheral leukocytes (Amisten et al., 2013; Matsumoto et al., 2000; Regard et al., 2008). Moreover, GPR27 is significantly enriched in the heat-gated cation channel-expressing ($TRPV1^+$) neurons from rat sensory ganglia (Isensee et al., 2014), the mouse gastric ghrelin cells (Engelstoft et al., 2013), blood from patients with meningeal injury (Livingston et al., 2017) and uncomplicated dengue (van de Weg et al., 2015). Gpr27 expression was also shown in infantile coho salmon acutely exposed to cadmium (Williams & Gallagher, 2013). In addition, two clinical reports found a genetic deletion of GPR27 (among other genes) in cases of developmental and speech delay, contractures, hypertonia and blepharophimosis (Pariani, Spencer, Graham Jr, & Rimoin, 2009; Petek et al., 2003). Furthermore, a promoter methylation mapping at 3p11.2-p14.2 in cervical cancer revealed that GPR27 displays a methylation-driven regulation pattern and may represent a new suppressor candidate (Lando et al., 2015). Finally, GPR27 is contained within a well-conserved alternative reading frame (ARF or matreshka) that may encode a yet unknown protein (Ribrioux, Brüngger, Baumgarten, Seuwen, & John, 2008). Whether the high conservation of this matreshka is responsible for maintaining the GPR27 protein sequence, or whether the opposite is the case, still needs to be clarified.

GPR27 has been shown to be a positive modulator of insulin transcription and secretion in vitro through a mechanism involving pancreatic and duodenal homeobox 1 (Pdx-1) (Ku, Pappalardo, Luo, German, & McManus, 2012). Furthermore, in an in vivo follow-up study, the authors showed that mice depleted for GPR27 exhibit 30% mRNA reduction of insulin and Pdx1 in the islets. This translated into a small effect on glucose tolerance (Chopra et al., 2020). This phenotype was recently corroborated in zebrafish where GPR27 deletion potentiated glucose elevation and abrogation of insulin-dependent Akt phosphorylation. Interestingly, in this model, medium-chain acylcarnitines, i.e. lipids known to be associated with insulin resistance in humans, were elevated (Nath et al., 2020).

GPR27 was recently suggested to be one of the GPCRs that mediate plasmalogen (glycerophospholipids characterized by the presence of vinyl ether linkage at the sn-1 position)-induced signaling in neuronal cells (Hossain, Mineno, & Katafuchi, 2016). However, the direct effect

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of GPR27 overexpression on signaling was not studied due to the author's inability to clone the mouse Gpr27. Additionally, these plasmalogens (Pls) are a mixture of 96.5% ethanolamine Pls, 2.5% choline Pls, 0.5% sphingomyelin and 0.5% other phospholipids. Therefore this imprecise composition is not really suited for proper pharmacological investigations, not to mention the difficulties in obtaining such reagents to confirm the data.

More recently, GPR27 was found to modulate hepatocellular carcinoma (HCC) progression. In this study, the authors observed that overexpression of the receptor led to an increase of various markers of proliferation while the knock-down of GPR27 had opposing effects. The effects were mediated by the MAPK/ERK pathway and the effect was confirmed in vivo. Overall, these data suggest a biological role of GPR27 in HCC development and progression (Wang et al., 2022).

In terms of signaling pathways, GPR27 was proposed to be constitutively coupled to the G protein families $G_{q/11}$ (Ku et al., 2012) and $G_{i/o}$ (Martin, Steurer, & Aronstam, 2015). More recently, we discovered surrogate ligands for GPR27 (see below Section 5) and used a pharmacological approach to interrogate GPR27 signaling pathways (Dupuis et al., 2017). We established that the activated receptor could interact with β-arrestin 2 but, in contrast with some literature, we could not find evidence of G protein signaling with these ligands. These observations may be the results of poor efficacy of the ligands for the G protein module but also indicate that GPR27 is an atypical, arrestin-biased receptor (Dupuis et al., 2017). These observations were recently partially corroborated by Lu et al. who investigated the constitutive activity and G protein coupling profile of various understudied orphan receptors. They used an assay consisting of the addition of GDP to disrupt spontaneous receptor-G protein complexes and monitoring of the dissociation with a BRET-based technique. They detected no significant coupling to G proteins for GPR27 or other SREBs (Lu, Jang, Inoue, & Lambert, 2021). The surrogate GPR27 agonists were recently used to investigate the function of GPR27 in astrocytes and 3T3 embryonic fibroblasts engineered by CRISPR/Cas9 to lack the receptor (Dolanc et al., 2022). In this report, the activation of GPR27 was linked to an increase of cytosolic L-lactate, the end product of aerobic glycolysis.

4.1.2. GPR85

Initial reports for GPR85 (SREB2) expression identified the presence of its mRNA in virtually all neurons of higher brain structures albeit with uneven expression levels (Matsumoto et al., 2005). In the brain, the highest levels of mRNA were observed in the thalamus, but also in the cerebellum, cerebral cortex, medulla, occipital pole, frontal and temporal lobe, putamen, amygdala, caudate nucleus, hippocampus, substantia nigra and Purkinje cells (Matsumoto et al., 2000; Yanai et al., 2016). Relatively low levels are found in corpus callosum, medulla and spinal cord. The mRNA copies were particularly abundant in regions characterized by high levels of neuronal plasticity such as the hippocampal formation, olfactory system, and supraoptic and paraventricular nuclei of the hypothalamus. In addition, in situ hybridization experiments performed on the human brain led to the detection of GPR85 mRNA in the granular cells of the hippocampus dentate gyrus (Matsumoto et al., 2005), known for its role in adult neurogenesis (Kempermann, Song, & Gage, 2015). There is also a high expression of the receptor during the development of the cerebral cortex in mice (Wittenberger, Schaller, & Hellebrand, 2001).

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Other tissues where GPR85 has been detected are the whole eye, pituitary gland, islets of Langerhans (Regard et al., 2008), ghrelin cells (Engelstoft et al., 2013), muscle-myenteric nerve layer (Ito et al., 2009), macrophages (Lattin et al., 2008) and osteosarcoma (Olstad et al., 2003).

The putative function of GPR85 has been mainly studied in the brain. Using transgenic GPR85-overexpressing or knockout mice, Matsumoto et al. observed that the receptor could indeed negatively influence brain size, impair hippocampal adult neurogenesis, neurogenesis-dependent learning and memory behavior (Chen et al., 2012; Matsumoto et al., 2008). An increase in vulnerability to schizophrenia was also linked to genetic variations of GPR85 human gene (Alavi, Shamsizadeh, Azhdari-Zarmehri, & Roohbakhsh, 2018; Matsumoto et al., 2008; Radulescu et al., 2013). In addition, several comparative genomics studies performed in humans identified the GPR85 gene or GPR85 locus (7q31) as possibly involved in Tourette syndrome and intellectual disability (Patel et al., 2011), attention deficit disorders (Anney et al., 2008) or autism (Voineagu et al., 2011). Interestingly, GPR85 has been shown to interact with postsynaptic density protein 95 (PSD-95) and neuroligin, proteins linked to autism spectrum disorder, further strengthening its potential role in the disease (Fujita-Jimbo et al., 2015).

4.1.3. GPR173

GPR173 (SREB3) expression analysis by northern blot on human tissues showed high levels throughout the brain (relatively high levels in cerebellum, hypothalamus, cerebral cortex) and ovaries, whereas lower levels were found in the small intestine (Matsumoto et al., 2000; Regard et al., 2008). Compared to GPR27 and GPR85, GPR173 is less present in the striatum (Matsumoto et al., 2005). It remains to be clarified whether the genomic location on a sex-determining chromosome is conserved for GPR173 as it may contribute to gonadal development, a critical feature in vertebrates (Livernois, Graves, & Waters, 2012). In addition, more than 450 fish species are hermaphroditic (Kuwamura, Sunobe, Sakai, Kadota, & Sawada, 2020) and a role of GPR173 is conceivable in that context.

In 2013, GPR173 was proposed to be the cognate GPCR mediating the effect of Gonadotropin-releasing hormone (GnRH) (1–5) on CN11 (immortalized GnRH neurons) cellular migration, a mechanism involving STAT3 (Larco, Semsarzadeh, Cho-Clark, Mani and Wu, 2013a, Larco, Semsarzadeh, Cho-Clark, Mani and Wu, 2013b). Although there are some indicators that this effect is G protein-mediated, no effects on cAMP or IP_3 levels could be observed; in contrast to β-arrestin 2, which was robustly recruited to GPR173 in these cells (Larco et al., 2013b). Therefore, the authors proposed a mechanism different from the canonical G proteins to explain the effect of GnRH(1–5) on cellular migration. Besides GnRH(1–5), phoenixin-20 amide has been suggested to mediate its effect through activation of GPR173 (Stein et al., 2016; Treen, Luo, & Belsham, 2016). The described stimulatory action on reproductive function, implicating up-regulated GnRH, GnRHR and Kiss1 genes, is explained by a GPR173-dependent action on the cAMP/protein kinase A pathway through CREB. A common point of both proposed ligands is the implication in the GnRH-related reproductive system. Whether GPR173 can bind these endogenous agonists needs to be independently demonstrated and clarified by further investigations, as well as elucidation of its exact signaling pathways. Of important note, the view of phoenixin being a ligand for GPR173 was recently

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challenged by Yanez-Guerra et al. that could not observe GPR173 signaling with this molecule (Yañez-Guerra, Thiel, & Jékely, 2022).

5. Synthetic ligands and pharmacological tools

Despite a couple of studies that have been published, the current lack of validated endogenous ligands for SREBs seriously limits the options to investigate the (patho)physiological functions of these receptors. Recently, we disclosed the results of the screen of ~7000 compounds from a diversity-oriented synthesis library (the DIVERSet from ChemBridge) on an assay designed to detect GPR27 activation (Dupuis et al., 2017). The strategy we followed was based on a split Firefly Luciferase whose two fragments were fused to the receptor and the β-arrestin 2. To further increase the sensitivity of the complementation assay, the C-terminal tail of GPR27 was replaced with the one of the vasopressin V_2 receptor that is known to bind β-arrestin 2 efficiently (Kroeze et al., 2015; Zindel et al., 2015). After the validation of our detection system with a membrane-targeted GRK2 that constitutively phosphorylates the receptor, we conducted the screening and identified two hit agonists, "5128535" and "5217941", with pEC_{50} values of 6.34 and 6.18, respectively, and a similar dichlorobenzamide-sulfonamide core (Fig. 3). These two hit compounds were discovered to be selective for GPR27V2 over GPR85V2 and GPR173V2. When tested on the unmodified receptor (without the V2 tail), both ligands induced the recruitment of β-arrestin 2 but failed to trigger G protein signaling in the assays we conducted (Dupuis et al., 2017).

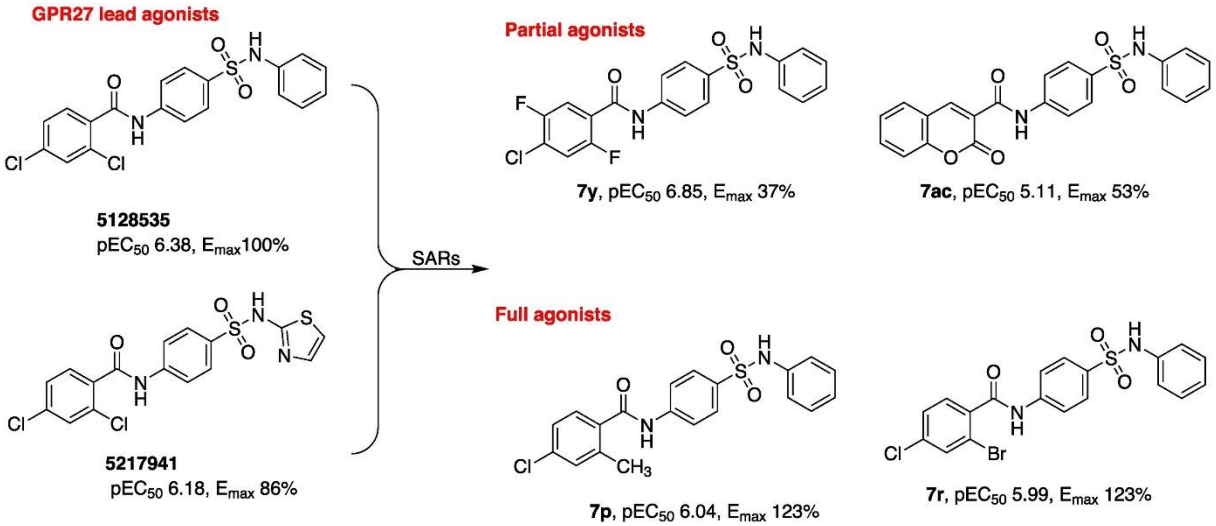

Fig. 3. Identification of GPR27 lead agonists 5,128,535 and 5,217,941 and the subsequent SAR study led to the full agonists (7p, 7r) that have greater efficacy compared to the reference. Other compounds (7y, 7 ac) have a lower efficacy compared to 5,128,535 and are labeled as partial agonists. The E_{max} of the different compound is indicated as a percentage of the E_{max} of 5,128,535 that is used as a reference. The E_{max} of 5,128,535 is arbitrarily set up at 100%.

These first GPR27 agonists served as a starting point for extensive structure-activity relationships. As a result, a variety of ligands with varying potencies and efficacies have been discovered, including partial (7y, 7ac) and full (7p, 7r) agonists with higher efficacies than the lead compounds (Fig. 3). The

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selected compounds were soluble and did not show any cellular toxicity at pharmacological concentrations. These tools might prove very useful for future research of GPR27 receptor function (Dolanc et al., 2022; Pillaiyar et al., 2021).

Prior to our findings, Yanai et al. described a series of non-selective inverse agonists for the SREB family generated from pyrazoles and coumarins (Yanai et al., 2016). To evaluate the activity of their chemical compounds, they employed the receptors fused with Gs (GPR27–Gs, GPR85–Gs and GPR173–Gs) and monitored the activity of their chimeras with a [^{35}S] GTPγS-binding assay. In our test system, however, the reported inverse agonists were unable to trigger GPR27-, GPR85- or GPR173-specific signals (Dupuis et al., 2017). These discrepancies could be related to the use of different assays and cell lines.

The same research group recently released a report on the discovery of novel SREB family ligands with improved inverse agonistic activity (Sakai et al., 2022). It is worth noting that the specificity of these inverse agonists against a different control receptor was not tested. In order to study these characteristics and validate the mechanism of action of this class of compounds, further research is required.

6. Conclusions and perspectives

GPR27, GPR85 and GPR173, collectively called "SREBs", constitute an atypical, highly conserved family of orphan GPCRs predominantly expressed in the brain. Their profile suggests important physiological functions but their role remains elusive and intriguing. Based on our current knowledge, different careful assumptions can be made.

First, GPCRs that form subfamilies tend to bind similar ligands and have overlapping functions. This is illustrated by clusters of similar size like the receptors for cannabinoids (Pertwee et al., 2010), the formyl-peptides (Ye et al., 2009), histamine (Alexander, Mathie, & Peters, 2006), leukotrienes (Bäck et al., 2014) or many others (Alexander et al., 2021). This rule may apply to SREB and one could speculate that they share similar or common ligands and overlapping functions. This assumption could explain the relatively mild phenotype in single-SREB whole knockout animals reported so far. In line with this hypothesis, the loss of one SREB would be compensated by one or both of the others, except in the tissues where they are not all present or not expressed at the same level or at the same time. For instance, a distinct pattern of expression of GPR27 and the other SREBs in the islet of Langherans would explain the recent data on the role of this receptor in glucose metabolism in vivo.

Secondly, the pattern of expression suggests a link between brain and peripheral functions, such as brain-metabolism, brain-reproduction or brain-immune system axis. The different mouse models that have been developed did not reveal such connections yet. However, the models that were reported are basic whole KO animals. Thus, these approaches could be improved by doing selective, conditional deletions and providing double or triple KO. Another aspect that should be considered is the limitations of mice as an experimental system. The highly controlled environment devoid of

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food or mate restrictions may mask relevant phenotypes. The absence of potent ligands limits also the in vivo investigations as the animals cannot be challenged with, for example, an agonist.

Thirdly, regarding the educated guess about the natural ligands, an interesting question is whether the ligands are necessarily conserved. As peptides and proteins are encoded by genes that are exposed to evolutionary pressure, the putative ligands are more likely to be small molecules that are not modified during evolution. There are no clear rules derived from the observations of other GPCRs. The receptors that bind small molecules are not more conserved compared to the receptors for peptides/proteins. Rather than a small molecule, the SREB could also bind highly conserved peptides/proteins, such as those found in the extracellular matrix. Another potential scenario is that the endogenous ligand, encoding a peptide or protein, co-evolved with its receptor, as it has been shown for luteinizing hormone (LH) and follicle-stimulating hormone (FSH) receptor (Moyle et al., 1994). A more recent study highlighted that peptide ligands and their respective GPCRs indeed coevolved, although the ligands were revealed as being more adaptive than the receptors (Foster et al., 2019). An important aspect that should be analyzed besides the discovery of the relevant ligand(s) is whether the binding site of the SREBs is also conserved or how it differs between receptors. This type of analysis will become feasible, in the absence of endogenous ligands, when surrogate ligands are available for all SREB members. Another possibility is that the ligand itself is encoded in the receptor, as it is the case for several other GPCRs (e.g. Proteinase-activated receptors or PARs, GPR52, TSHR). Fourthly, the high degree of conservation of SREBs throughout vertebrate evolution suggests a role in processes like ontogenesis or development of tissue architecture, like it is for instance known for the receptors of the frizzled family, which are also highly conserved. One further important line of thought that could feed future endeavors is to nail down a function that is shared by all SREBs and is specific to vertebrates, such as in the neuroendocrine or reproductive systems.

Definitions

Homologs (Moreira, 2014a) or homologous genes are genes that share a common evolutionary origin. These genes include both, genes that arose from speciation events (orthologs) and genes that arose from duplication events (paralogs).

Orthologs (Moreira, 2014b) or orthologous genes are genes originating from a common ancestral gene by speciation. Thus, they are present in different species and usually retain similar functions in these different species during evolution

Paralogs (Moreira & López-García, 2014) or paralogous genes are genes within one species originating from gene duplication. Paralogous genes may keep the same function but more often diverge and develop different functions. Further, paralogs can be retained in the genome but some copies may also be lost during evolution.

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Declaration of Competing Interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

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Abbreviations

βAR, β-Adrenergic receptors; β2AR, β2-Adrenergic receptor; β3AR, β3-Adrenergic receptor; D2, Dopaminergic receptor 2; D4, Dopaminergic receptor 4; ECL, Extracellular loop; EST, Expressed Sequence Tags; FSH, Follicle-Stimulating Hormone; FZD, Frizzled receptors; GnRH, Gonadotropin-releasing hormone; GPCR, G protein-coupled receptors; GRK, G protein-coupled receptor kinase; H1, Histamine receptor 1; HCC, Hepatocellular carcinoma; 5HT1A, Serotonin receptor 1A; ICL, Intracellular loop; IUPHAR, International Union of Basic and Clinical Pharmacology; LH, Luteinizing Hormone; M2,MusacarinicM2 receptor; MC4R,Melanocortin receptor 4; mPFC, medial Prefrontal Cortex; PSD-95, postsynaptic density protein 95; RHO, Rhodopsin; RXFP, Relaxin family Receptor; SH3, Src-homology 3; SHANK, SH3 And Multiple Ankyrin Repeat Domains; SREB, Super Conserved Receptors Expressed in the Brain; TM, Transmembrane.

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