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Insight into SUCNR1 (GPR91) structure and function

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ABSTRACT

SUCNR1 (or GPR91) belongs to the family of G protein-coupled receptors (GPCR), which represents the largest group of membrane proteins in human genome. The majority of marketed drugs targets GPCRs, directly or indirectly. SUCNR1 has been classified as an orphan receptor until a landmark study paired it with succinate, a citric acid cycle intermediate.

According to the current paradigm, succinate triggers SUCNR1 signaling pathways to indicate local stress that may affect cellular metabolism. SUCNR1 implication has been well documented in renin-induced hypertension, ischemia/reperfusion injury, inflammation and immune response, platelet aggregation and retinal angiogenesis. In addition, the SUCNR1-induced increase of blood pressure may contribute to diabetic nephropathy or cardiac hypertrophy.

The understanding of SUCNR1 activation, signaling pathways and functions remains largely elusive, which calls for deeper investigations. SUCNR1 shows a high potential as an innovative drug target and is probably an important regulator of basic physiology. In order to achieve the full characterization of this receptor, more specific pharmacological tools such as small-molecules modulators will represent an important asset. In this review, we describe the structural features of SUCNR1, its current ligands and putative binding pocket. We give an exhaustive overview of the known and hypothetical signaling partners of the receptor in different in vitro and in vivo systems. The link between SUCNR1 intracellular pathways and its pathophysiological roles are also extensively discussed.

Contents

Abbreviations: (GPCRs), G protein-coupled receptors family; (SUCNR1), succinate receptor 1; (AA), amino acids; (7TM), seven transmembrane domains; (ECLs), extracellular loops; (ICLs), intracellular loops; (cAMP), cyclic adenosine monophosphate; (AC), adenylate cyclase; (GRKs), G protein-coupled receptor kinases; (CHO) cells, Chinese hamster ovary; (HEK293) cells, human embryonic kidney; (MDCK) cells, madin darby canin kidney; (iDC), immature dendritic cells; (PTX), pertussis toxin; (ERK1/2), extracellular signal-regulated kinases 1 and 2; (RGC), retina ganglion cells; (RAS), renin–angiotensin system; (JGA), juxtaglomerular apparatus; (MD), macula densa; (JG) cells, juxtaglomerular; (COX-2), cyclooxygenase 2; E2 (PGE2), prostaglandin; (PGI2), prostaglandin I2; (AA), arachidonic acid; (GENCs), juxtaglomerular endothelium cells; (NO), nitric oxide; (CH), cardiac hypertrophy; (CaMKIIδ), calcium/calmodulin-dependent protein kinase IIδ; (HDAC5), histone deacetylase 5; (PKA), protein kinase A; (PLN), phospholamban; (RyR2), ryanodine receptor 2; (PDE), phosphodiesterase; (RVH), right ventricular hypertrophy; (LVH), left ventricle hypertrophy; (PI3K), phosphatidylinositol-4,5-bisphosphate 3-kinase; (Akt), protein kinase B; (WAT), white adipose tissue; (MS), metabolic syndrome; (ASA), acetylsalicylic acid; (ADP), adenosine diphosphate; (TXA2), thromboxane A2; (DR), diabetic retinopathy; (VEGF), vascular endothelial growth factor; (JNK), c-Jun N-terminal kinases; (ROP), retinopathy of prematurity; (CHI), cerebral hypoxic–ischemic; (EP_4), prostaglandin E receptor 4; (AMD), age-related macular degeneration; (RPE), retinal pigment epithelium; (FSK), forskolin; (HSC), hepatic stellate cells; (α-SMA), α-smooth muscle actin; (HPC), hematopoietic progenitor cells; (IP), inositol phosphate; (iDC), immature dendritic cells; (TNF-α), tumor necrosis factor α; (IL-1β), pro-inflammatory cytokine interleukin-1 beta; (IFN-γ), interferon γ; (IRI), ischemia–reperfusion injury.

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1. Succinate Receptor 1 structure and ligands

SUCNR1 was first spotted in a megacaryocytic cell line in 1995 and called "$P2_{U2}$", a name coined for its homology with the purinergic receptor $P2Y_2$, known as $P2_U$ at that time (Gonzalez et al., 2004). *SUCNR1* gene was later re-discovered as *GPR91* in 2001 on human chromosome 3q24–3q25 using an expressed sequence tag data mining strategy (Wittenberger et al., 2001). Of important note is the possibility of two open-reading frames (ORF) for *SUCNR1*, one giving a protein of 330 amino acids (AA) and the other one 334 AA. Wittenberger et al. noted that the 330-AA protein was more likely to be expressed given the Kozak sequence surrounding the second ATG (Wittenberger et al., 2001). In the present article we will use the AA numbering according to a 330-AA protein, although the current databases sometimes report SUCNR1 as being 334-AA long. There is a high degree of homology between man and mouse (68%) with the exception of the C-terminal tail, which is 12 AA shorter in rodents (Ariza et al., 2012; Wittenberger et al., 2001). In humans, other genes have been found on the same locus and consist in a cluster of *$P2Y_1$, $P2Y_{12}$, $P2Y_{13}$, H963* (*GPR171*) and *GPR87* (Abbracchio et al., 2006). In their seminal paper, Wittenberger et al. speculated that all these receptors originated from a common ancestor, presumably a nucleotide receptor (Wittenberger et al., 2001). Several comprehensive reviews have been published on the receptor or succinate role in metabolic/oxidative stress conditions (Ariza et al., 2012; Peti-Peterdi et al., 2013). The present work, in addition to discussing the most recent developments, considers extensively the mechanisms linking SUCNR1-activated signaling pathways and all (patho)physiological states where the receptor might play a role.

SUCNR1 belongs to G protein-coupled receptors (GPCRs) family. In the human genome, it is the largest group of proteins involved in signal transduction across biological membranes (Fredriksson et al., 2003). GPCRs are currently the direct or indirect target for ~60% of marketed drugs and thus the most successful receptor family for treating human diseases (Davenport et al., 2013). GPCRs are classified into different families according to sequence homology and to their various types of ligands. Rhodopsin-like or class A GPCRs can be sub-divided in four groups: α, β, γ and δ. SUCNR1 belongs to the latter (Fredriksson et al., 2003) and possesses a number of highly conserved residues and short-sequence motifs (Venkatakrishnan et al., 2013; Wittenberger et al., 2001). SUCNR1 was initially viewed as a purinergic receptor due to its high sequence homology with P2Y receptors (29% with $P2Y_1$ (Wittenberger et al., 2001)) and predicted to bind purinergic ligands (Fredriksson et al., 2003; Joost & Methner, 2002; Wittenberger et al., 2002). However, it has been paired by He et al. with a molecule not even remotely similar to purines: succinate (He et al., 2004).

Receptors responding to purines and derivatives are classified between ionic channels P2X and metabotropic receptors (GPCRs) P2Y. In 2006, the P2Y family was divided between $P2Y_1$-like receptors and $P2Y_{12}$-like receptors based on three criteria (Abbracchio et al., 2006). First, phylogenetic similarity, second the presence of AA motifs proposed to be important for ligand binding, and, third, primary G protein coupling. Regarding the second criterion, SUCNR1 has some interesting similarities with $P2Y_1$-like receptors in the TM6 (see below) such as the $H^{6.52}XXR/K^{6.55}$ and a slightly modified Q/KXXR (SUCNR1 has $IVTR^{7.38}$) motifs (*superscript indicates residue numbering using Ballesteros–Weinstein nomenclature* (Ballesteros & Weinstein, 1995)). They might be important for agonist activity. For the third criterion, SUCNR1, just like $P2Y_{12, 13, 14}$ receptors, almost exclusively couples to $G_{i/o}$ (see below) in contrast with $P2Y_1$-like receptors that preferentially activate G_q signaling and induce calcium release (Abbracchio et al., 2006). Therefore, with regard to purinergic receptor classification, SUCNR1 cannot be related to either class, although it has striking similarities with this family.

A better understanding of SUCNR1 structure will be a key step towards development of potent small-molecules modulators. Little information is currently available concerning the receptor tridimensional structure. Recently, many crystallographic data for class A GPCRs bound to different ligands, in different crystal forms or using different approaches to receptor stabilization and crystallization have been disclosed (a complete listing is outside the scope of this article but readers may find complete information in recent reviews (Katritch et al., 2013; Zhang et al., 2015)). Although SUCNR1 has not been crystallized yet, the information on its structure can be hypothesized by comparison with closely related proteins. Two representative purinergic receptors ($P2Y_1$ and $P2Y_{12}$) have been crystallized recently (Zhang et al., 2015; Zhang J. et al., 2014; Zhang K. et al., 2014) and some careful inferences can be made on SUCNR1 structure.

1.1. Extracellular domains

SUCNR1 shares with GPCRs the general extracellular structure where transmembrane domains are connected by three hydrophilic extracellular loops (ECLs). A disulfide bond highly conserved among GPCRs links the top of the TM3 (at the end of ECL1) to the middle of ECL2 and is present in many GPCRs. The presence of two conserved cysteines in ECL2 (C168) and top of TM3 ($C91^{3.25}$) suggests that SUCNR1 possesses this canonical disulfide bridge (Fig. 1).

A second conserved disulfide bridge is often observed between the N-terminus of the receptors and the top of TM7 (or at the end of ECL3). It stabilizes the extracellular structures and forms a pseudo-loop that has recently been defined as "ECL4" (Szpakowska et al., 2014). This feature is present in both $P2Y_{12}$ and $P2Y_1$ crystal structures (Zhang et al., 2015; Zhang J. et al., 2014). It is tempting to speculate that SUCNR1 has a similar architecture since it has two cysteines positioned at topologically similar positions (C7 and $C264^{7.26}$). Resolution of SUCNR1 crystal structure is expected to confirm the presence of the disulfide bonds and mutations of these residues should show if the bridges are critical for interactions with ligands.

SUCNR1 has two N-glycosylation sites, at N^8 and N^{168} in the N-terminus and in the second extracellular loop, respectively (Robben et al., 2009; Wittenberger et al., 2001). However the precise role of this post-translational modification remains unknown. These post-translational modifications have been well characterized in some members of the P2Y family. For example, N-linked glycosylation of $P2Y_{12}$ receptors is essential for signal transduction (Zhong et al., 2004). In SUCNR1, there is also one phosphorylation site that might be important for the receptor internalization, i.e., S^{326} in the C-terminus (Fig. 1) (Wittenberger et al., 2001).

1.2. Transmembrane domains and binding pocket

SUCNR1 shares the classical features of class A receptors together with some typical characteristics of purinergic receptors.

1.2.1. TM1–TM2

$N^{1.50}$ residue in TM1, together with the TM2 $L^{2.46}XXXD^{2.50}$, are implicated in interhelical hydrogen bonding (Venkatakrishnan et al., 2013; Wittenberger et al., 2001).

1.2.2. TM3

The conserved $E/DR^{3.50}Y$ motif plays an important role in controlling GPCR activation (Audet & Bouvier, 2012; Rovati et al., 2007). Current paradigm proposes that $R^{3.50}$ participates in an "ionic lock" with a negatively charged glutamate ($E^{6.32}$) in TM6. Studies have shown that disruption of this interaction leads to TM6 movement away from the TM bundle to facilitate interaction with G protein, suggesting a common conserved mechanism for receptor activation induced by ligand binding (Audet & Bouvier, 2012). Simultaneously, the $Y^{7.53}$ of the conserved $NP^{7.50}XXY$ motif moves inside the bundle, blocking TM6 in an open conformation (Audet & Bouvier, 2012).

However this "ionic lock" is not formed in all GPCR structures co-crystallized with an antagonist. Like $P2Y_{12}$, SUCNR1 bears a $V^{6.42}$