Nuclear G-protein-coupled receptors as putative novel pharmacological targets

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Cell surface G-protein-coupled receptors (GPCRs) are targets for ~ 30% of drugs currently on the market, and are the largest group of gene products targeted by drugs. Until recently, signaling mediated by GPCRs was thought to emanate exclusively from the cell membrane as a response to extracellular stimuli. However, recent research has revealed the existence of nuclear (n)GPCRs with the ability to trigger identical and/or distinct signaling pathways to their respective counterparts on the cell surface. Understanding of the GPCR signaling platform on the nuclear membranes and its involvement in physiology and/or pathophysiology will be important to develop selective pharmacological and pharmaceutical approaches. In this review, we summarize our current understanding of nGPCRs, with emphasis on their potential as novel pharmacological targets.

Introduction

GPCRs belong to a diverse superfamily with ~ 800 members with heptahelical transmembrane domains [1]. According to the *IUPHAR Guide to Pharmacology*, GPCRs can be divided into six classes (A–F) based on their sequence homology: A (rhodopsin-like); B (secretin receptor family); C (metabotropic glutamate); D (fungal mating pheromone receptors); E (cyclic AMP receptors); and F (frizzled), with classes D and E not found in vertebrates [1].

Conventionally, GPCRs are cell surface receptors (mGPCRs) that respond to a variety of external stimuli through ligands including peptides or proteins, ions, lipids, odorants and light. GPCRs signal commonly through the heterotrimeric guanine (G) nucleotide-binding proteins (α, β, and γ subunits), mediating and dictating their downstream signaling and ultimate effects [2]. Over the past few decades, an increasing body of evidence has highlighted the existence of active GPCR signaling systems in intracellular compartments, including endosomes, endoplasmic reticulum (ER), mitochondria, and nucleus [3]. Some have been intensively studied, revealing attractive physiological functions and/or plausible roles in pathological conditions. Therefore, the occurrence of GPCRs other than mGPCRs requires a reassessment of, and development of new, models for pharmacological interventions. This is particularly important given that GPCRs are targets for approximately one-third of drugs used in clinical practice, acting on 108 GPCRs [4]. In this review, we provide a summary of the current knowledge regarding nGPCRs and discuss their potential as pharmacological targets for new and of already used drugs.

Nuclear GPCRs: from origin to function

In 1971, a pioneering study demonstrated that injection of angiotensin II (Ang II) in a nuclear zone induced ultrastructural cellular changes [5], an effect later attributed to the triggering of a GPCR located in the nucleus. Since then, evidence supporting the nuclear localization of GPCRs has been acquired mainly through the use of

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https://doi.org/10.1016/j.drudis.2019.09.003

radioligand binding assays, electron and confocal microscopy techniques [immunolabeling, fluorescent ligands or receptors fused with fluorescent tag(s)], and western blots [6], using isolated nuclei and/or intact cells, and functional assays with caged and/or photolyzed ligands [7] or cytosolic microinjections [6].

More than 40 functional nGPCRs from several classes have been identified in the nuclear membrane of distinct cell types, as summarized in Table 1. Despite extensive research efforts, further clarification is required for some aspects of nGPCRs, including their exact nuclear location, relevant receptor and ligand origins, signaling cascades and ultimate effects. Nevertheless, nGPCRs appear to be evolutionarily conserved, given that their presence has been confirmed in a range of organisms, including nematodes (*Caenorhabditis elegans*), insects (*Drosophila*), and mammals, as also summarized in Table 1.

Origin of nuclear GPCRs

The appearance of GPCRs in the nucleus has been suggested to have occurred in at least four different ways (Fig. 1). nGPCRs could appear in the nucleus following mGPCR internalization and their subsequent translocation into the nuclear membrane [8]. Multiple mechanisms for mGPCR nuclear translocation (from cell membrane to nucleus) have been reported [8], via a process that can be either agonist dependent or independent. Agonist-dependent translocation of GPCRs occurs as a consequence of prolonged and/or repetitive exposure to agonists. In some cases, the receptor can be internalized along with the concomitant activation of signal pathways, such as the mitogen-activated protein kinase (MAPK) and protein kinase B (Akt) and/or phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathways [9,10], which elicit genomic delay responses [11]. Once internalized, GPCRs can be recycled back to the cell membrane or transported to organelles such as lysosomes, ER, Golgi body [12–14], and mitochondria [3] or, ultimately, translocated into nucleus [3,11,15]. The GPCRs translocated from the cell membrane to the nucleus can (but not always) trigger a late response (e.g., gene transcription) which can complement (or oppose) the initial response triggered by mGPCR, without retaining the information obtained from the extracellular space.

Other stimuli or events can promote mGPCR nuclear import from the cell membrane via an agonist-independent process. Such GPCR activation challenges traditional GPCR pharmacology and introduces the environment as an important, and in some cases, decisive factor determining certain signal events and effects. For example, the neurokinin 3 (NK_3) receptor is activated, internalized, and transported to the nucleus following osmotic changes [16]. Other examples include the presence of parathyroid hormone 1 (PTH1) receptors in nuclear membranes of osteoblast-like cells only during certain stages of the cell cycle, when the DNA is exposed to transcriptional activity [17], and the presence of the vasoactive intestinal peptide 1 ($VPAC_1$) receptor, the translocation of which is induced by palmitoylation [18].

Agonist-dependent or -independent nuclear translocation appears to be cell type specific and depends on cellular conditions, such as concomitant activation with other receptors, metabolic state, or pathologies. The oxytocin (OT) receptor (OT [19]) and Ang II receptor type 1 (AT_1 [20,21]) have demonstrated both agonist-dependent and -independent nuclear localizing capabilities according to the cell type. Any alteration of any of the environmental conditions would lead to a modified response triggered by nGPCRs.

The translocation of GPCR to the nucleus has a specific fingerprint: in the C terminus or an intracellular loop of nGPCRs, short peptide sequences comprising basic amino acid residues (usually lysine/glycine-arginine repeats) have been found and are considered classical nuclear localization sequences (NLS) [22]. These NLS appear to direct GPCRs to the nucleus via small GTPases and/or importin mechanisms, as demonstrated for platelet-activating factor (PAF) [23], OT [11], protease-activated 2 (PAR2) [24], and PTH1 receptors [25].

The translocation of receptors might be independent on the presence of the NLS motive, as observed for the translocation of the bradykinin B_2 receptor, which, despite the NLS being present, is dependent on heterodimerization of the receptor with lamin C [26]. Furthermore, other peptide sequences that do not resemble classic NLS might also promote the nuclear import of receptors [27]. For example, the M9 sequence, a 38-amino acid-long fragment from heterogeneous nuclear ribonucleoproteins A1 and A2 proteins, is recognized by transportin, which mediates both nuclear import and export [28].

In addition to the cell membrane origin of nGPCRs, recent findings have suggested that resident nGPCRs are synthesized in ER (in an identical process to that described for mGPCRs) and traffic directly to the nucleus by lateral diffusion, as appears to occur with the endothelin subtype B (ET_B) receptor [29]. Other recent findings also suggest that GPCRs also appear in the nucleus through a fourth pathway: the direct synthesis of nGPCRs within the nucleus [30], a possibility supported by the occurrence of protein translation inside the nuclei of mammalian cells, supporting this as a possible direct source of nGPCRs [8,31].

Location and function of nuclear GPCRs

nGPCRs have been found in the nuclear envelope at the inner and/or outer nuclear membranes (INM and ONM, respectively) where they can be anchored securely (as demonstrated for the metabotropic glutamate 5 (mGlu5) receptor, which is firmly retained in the INM via its interactions with chromatin [32]) and oriented in such a way that exposes the effector-binding domain to either the nucleus or the cytosol (Fig. 2). nGPCRs have also been suggested to be present in the nucleoplasm in the network of invaginations of both nuclear membranes [33] or through transport within micelles, maintaining membrane-embedded GPCR [34]. However, the nuclear location of GPCRs does not suggest that nuclear signaling is limited to the nucleus. Evidence suggests the presence of receptors in the INM with the effector-binding domain directed towards the interior of nucleus, but with ultimate effects in the cytosol because of the translocation of effector proteins to the cytosol, as observed for the α_{1A}-adrenoceptor [35].

Key components of signaling pathways and the machinery typically associated with mGPCRs (G proteins, downstream effector molecules, second messengers, ion channels, and regulators) are found in the nucleus of several cell types [3,6]. These data reinforce the idea that the nucleus is an autonomous signaling organelle retaining functional nGPCRs. nGPCRs mainly regulate nuclear calcium $[Ca^{2+}]_n$, nitric oxide levels or cAMP production, although other second messengers, such as IP3, cGMP, and DAG,

TABLE 1

Nuclear GPCR subtypes in different cells and/or tissues and their respective functions

Family name	Subtype(s)	Cell/Tissue	Signalling/Function	Refs
Class A				
Adrenoceptors	α_1	Mouse cardiac myocytes	$G\alpha_q$–PLCβ1–ERK activation	[36]
	α_{1A}	Mouse cardiac myocytes	α_{1A}–$G\alpha_{q/11}$–PKCδ–"cTnI contractility"	[35]
	α_1, β_1	Rat ventricular myocytes		[6]
	β	Rat cardiomyocytes	$G\alpha_i$–PI3K–Akt–ERK1/2–↓ NF-κB transcription; ↑ Ripk2 + ↓ NF-κB, ATF-2, IL1r1 and Tnfrsf1b transcription	[22]
	β_3	Rat cardiomyocytes	$G\alpha_i$–NOS–GC–PKG transcription	[22]
	β_1, β_3	Rat and mouse ventricular cardiomyocytes	β_1–"AC–↑ cAMP transcription induction"; β_3–"$G\alpha_i$ transcription induction"	[6]
Angiotensin receptors		Rat hypothalamus, thalamus, septum, and midbrain		[56]
		Rat VECs, SMCs, and cardiomyocytes		[5]
		Rat liver and spleen		[57]
		Rat liver	↑ Renin + angiotensinogen transcription	[6]
		Rat and hamster myocytes	↑$[Ca^{2+}]_n$	[6]
	AT_1	Rat liver		[6]
		Rat and human VSMCs	↑ $[Ca^{2+}]_n$	[6]
		Human VSMCs	↑ $[Ca^{2+}]_n$	[6]
		Rat VSMC line A10	↑ p-CREB; ↑ proliferation	[6]
		Transfected HEK293 cells, mouse cardiac myocytes	↑ *COX2* gene transcription (PTGS-2)	[21]
		mRen(2).Lewis rat kidneys	↓ Nuclear AT_1	[51]
		Rat kidneys	PKC–NADPH oxidase (NOX4) activation; ↑ ROS	[70]
	AT_1, AT_2	Rat and mouse substantia nigra pars compacta (dopaminergic neurons), transfected MES 23.5 dopaminergic neuron cell line	AT_1–IP3–↑ $[Ca^{2+}]_n$–↑ AT_2 + angiotensin + PGC-1α + IGF-1 transcription → protective effect; AT_1–NOX4–↑ superoxide/H_2O_2; AT_2–NOS–↑ NO	[71]
		Rat liver	↑ Renin, angiotensinogen, c-myc, PDGF transcription	[6]
		Rat cardiomyocytes	AT_1/AT_2–↑ NF-κB expression; AT_1–"IP3–↑ $[Ca^{2+}]_n$ transcription"	[22]
		Sheep kidney	AT_2–↑ NO	[72]
		Sheep kidney of a fetal programming model	↑ ROS, NO	[73]
		Canine cardiac fibroblasts	AT_1–"IP3–↑ $[Ca^{2+}]_n$"; AT_2–↑ NO"; AT_1+AT_2 regulate fibroblast proliferation, collagen gene expression, and collagen secretion	[7]
Apelin receptor		Human cerebellum and hypothalamus, Purkinje cells and transfected D283 Med cells		[34]
Bradykinin receptors	B_2	Rat hepatocytes	↑$[Ca^{2+}]_n$–"Akt acetylation of histone H3–iNOS gene induction"	[22]
	B_2	Transfected HEK cells	Heterodimer with lamin C	[26]
	B_2	Rat olfactory bulb, cerebral cortex, hippocampus, basal forebrain, basal ganglia, thalamus, hypothalamus, cerebellum, and brainstem		[58]
	B_2	Human placenta		[74]
	B_2	HEK-293T cells		[34]
	B_1, B_2	Rat hippocampus		[59]
Chemokine receptors	CCR2	Transfected HEK cells		[15]
	CXCR4	Human non-small cell lung cancer		[52]
		Human hepatoma cancer cells		[6]
		Human prostate cancer cell lines	$G\alpha_i$–↑ $[Ca^{2+}]_n$	[22]
		HeLa cells		[6]
		Human nasopharyngeal carcinoma		[75]
Endothelin receptors		Rat liver		[6]
		Human endocardial endothelial cells and aortic VSMCs	↑$[Ca^{2+}]_n$–↑ ROS	[22]
		Rat heart	↑NO–↓ transcription	[76]
	ET_B	Human heart and VSMCs	↑$[Ca^{2+}]_n$	[6]
	ET_B	Rat cardiomyocytes	IP3–↑ $[Ca^{2+}]_n$	[29]
	ET_A, ET_B	Rat cardiac ventricular myocytes	↑$[Ca^{2+}]_n$	[42]
Formylpeptide receptors	FPR2	Human cancer cells	$G\alpha_i$–ERK2, c-Jun, and c-Myc phosphorylation	[22]
Ghrelin receptor		Transfected HEK293 cells		[77]

TABLE 1 (*Continued*)

Family name	Subtype(s)	Cell/Tissue	Signalling/Function	Refs
Gonadotrophin-releasing hormone receptors	$GnRH_1$	HEK 293 and HTR-8/Svneo cell lines	Histone H3 acetylation and phosphorylation	[22]
	Ce-GnRHR	*Caenorhabditis elegans*		[6]
Leukotriene receptors	$CysLT_1$	Human colon epithelial cells and colon cancer cells	Proliferative ERK1/2 signaling	[22]
		Human VSMCs	↑ $[Ca^{2+}]_n$–PAI-2 transcription	[22]
	$CysLT_1$, $CysLT_2$	Mouse B16, human HaCaT prekeratinocytes	NOX4 nuclear translocation, ROS accumulation, and oxidative DNA damage	[78]
Lysophospholipid receptors	LPA_1	Porcine cerebral microvascular endothelial cells	COX-2 and iNOS transcription	[22]
		Transfected HTC4 rat hepatoma cells		[6]
		Rat liver	$G\alpha_{i/o}$–PI3K–Akt–↑ $[Ca^{2+}]_n$/↑ iNOS expression	[6]
	LPA_1	Porcine cerebral endothelial cells	GC–eNOS/cGMP phosphorylation–↑ $[Ca^{2+}]_n$ + p42 MAPK activation, NF-kB binding to DNA + iNOS expression	[6]
	LPA_1	HBEC, PC12, and CHO cells	Regulates protein phosphorylation	[6]
	$S1P_1$	C57BL/6 mouse splenic CD4 T cells	$G\alpha_{i/o}$–↓ p-Erk + p-c-Jun	[46]
	$S1P_1$	Human umbilical vein endothelial cells	Cyr61 and CTGF expression	[79]
Melanocortin receptors	MC_2	H295R cell	Interaction with Nup50	[6]
Melatonin receptors	MT_2	Human placental choriocarcinoma cell lines		[6]
Muscarinic Acetylcholine Receptors		Rabbit cornea and endothelial cells	↑ cGMP, ↓ cAMP	[6]
		Chinese hamster ovary cells	↑ DNA and RNA polymerase II activity	[6]
Neuropeptide Y receptors		Rat pituitary gland		[6]
	Y_1	Human endocardial endothelial cells	↑ $[Ca^{2+}]_n$	[6]
Neurotensin receptor	NTS_1	Rat substantia nigra, transfected Chinese hamster ovary cells, and human lung cancer cell line		[80]
		Rat brain		[81]
Opioid receptors	**δ**	NG 108-I 5 neurohybrid cells		[6]
	μ	Human mesothelial cell line and human abdominal adhesions		[82]
	k	Mouse undifferentiated GTR1 cells	PKC–GATA-4 + Nkx-2.5 gene transcription	[83]
	k	Pluripotent embryonic P19 cell line	GATA-4 + Nkx-2.5 gene expression	[6]
	k	Hamster ventricular myocardial cells	PKC–opioid peptide gene transcription	[22]
Oxytocin receptors	OT	Mouse osteoblasts	Expression of osteoblast differentiation genes (*Sp7*, Atf4, *Ibsp*, and *Bglap*)	[11]
		Human osteosarcoma (U2OS, MG63, OS15, and SaOS2), breast cancer (MCF7), and primary human fibroblastic cells (HFF)		[19]
		Human epithelial glandular cells of endometrium, peritoneal endometriosis, and endometriotic ovarian cysts		[84]
Platelet-activating factor receptor	PAF	Rat liver	Phospholipase C–DAG	[6]
		Porcine cerebral microvascular endothelial cells and neurons	$G\alpha_{i/o}$–↓ cAMP; ↑$[Ca^{2+}]_n$; ERK1/2 + NF-**κ**B binding–iNOS + COX-2 transcription	[22]
		HEK293T and CHO-K1 cells and rat retinas	↑ Growth factors (e.g., VEGF) → angiogenesis	[23]
Prostanoid receptors	EP_1	Porcine cerebral microvascular endothelial cells		[6]
		Transfected HEK 293 cells		
		Murine fibroblast Swiss 3T3 cells (overexpressing EP1)	↑ $[Ca^{2+}]_n$–c-fos transcription	
		Pig myometrium	↑ $[Ca^{2+}]_n$–c-fos transcription	
	EP_1, EP_3	Rat cerebral cortex endothelial cells and neurons		
	EP_3	Piglet brain endothelial cells	$G\alpha_{i/o}$–K_{Ca2+} Channels–↑ $[Ca^{2+}]_n$–PI3K/Akt + Erk1/2 + NF-**κ**B–↑ eNOS expression	[22]
	EP_1, EP_2, EP_3, EP_4	Neonatal porcine brain		[22]
		Rat liver	EP_3–$G_{i/o}$–↑ $[Ca^{2+}]_n$	
		Porcine cerebral microvascular endothelial cells	↑ iNOS expression	
	TP	Rat oligodendrocytes	$G\alpha_s$ -↑cAMP–p-CREB + myelin basic protein transcription + ↑ survival	[39]
	TP	Rat oligodendrocytes progenitor cells	↑ Myelin basic protein expression	[85]

TABLE 1 (*Continued*)

Family name	Subtype(s)	Cell/Tissue	Signalling/Function	Refs
Proteinase-activated receptors/ coagulation factor II (thrombin) receptor-like 1	F2rl1/PAR2	Mouse retinal ganglion cells	Sp1 recruitment–↑ VEGFα expression → neovascularization	[47,24]
Tachykinin receptors	NK_1	Rat dorsal root ganglia		[6]
	NK_3	Rat ventral tegmental area		[6]
	NK_3	Rat hypothalamus		[16]
Urotensin receptor	(UT)	Rat brain	↑ Total transcription rate	[44]
		Monkey brain, human glioblastoma-astrocytoma U87-MG and human neuroblastoma SH-SY5Y cell lines		
		Rat and monkey heart (except right ventricle)	Transcription initiation	[38]
Class B				
Parathyroid hormone receptors	PTH1	Red deer osteoclasts		[6]
	PTH1	Mouse osteoblast-like cell line (MC3T3-E1)		[20]
	PTH1	Osteoblast-like cells (mouse MC3T3-E1, rat ROS 17/2.8 and UMR106, and human SaOS-2)	In MC3T3-E1 cells, receptors are involved in DNA synthesis and mitosis	[86]
	PTH1	Rat liver, kidney, uterus, gut, and ovary		[6]
VIP and PACAP receptors	VPAC	Human colonic adenocarcinoma cell line (HT29)		[6]
	$VPAC_1$	Human breast carcinoma cell lines (T47D and MDAMB-468)	$G\alpha_s$ –↑ cAMP	[22]
	$VPAC_1$	Chinese hamster ovary cells	Antiapoptotic activity	[18]
	$VPAC_1$, $VPAC_2$, PAC_1	Glioblastoma multiforme cell lines		[48]
Class C				
Metabotropic glutamate receptors	$mGlu_1$	Rat cortex, olfactory bulb, thalamus, and cerebellum, transfected HEK293 cells	↑ $[Ca^{2+}]_n$; control of brain development	[6]
	$mGlu_5$	Heterologous cells, mouse midbrain and cortical neurons, transfected HEK293 cells	↑ $[Ca^{2+}]_n$	[22]
		Rat striatal neurons	↑ $[Ca^{2+}]_n$ + p-CREB	[22]
		Rat and monkey substantia nigra		[87]
		Rat striatal neurons, HEK293 cells	$G\alpha_{q/11}$–PLC–IP3/ryanodine receptor –↑ $[Ca^{2+}]_n$	[6]
		Neonatal rat and mouse striatal cells	JNK, CaMK, CREB, ERK1/2 and Elk-1 phosphorylation; p-Elk-1–↑ c-fos + egr1	[6]
		Rat and mouse striatal neurons	$[Ca^{2+}]_n$ + ERK1/2 + CaMK–↑ cytoskeletal-associated proteins (Arc/Arg3.1)	[45]
		Rat hippocampus	↑ $[Ca^{2+}]_n$	[37]
		Rat spinal dorsal horn neurons	↑ $[Ca^{2+}]_n$; p-ERK1/2, Arc/Arg3.1 and c-fos → neuropathic pain	[50]
Orphans Class Frizzled	GPR158	Human trabecular meshwork cell line	Cell proliferation	[22]
	FZD_2 (C-terminal)	*Drosophila* muscle (neuromuscular junctions)	Promotes postsynaptic development of subsynaptic reticulum/postsynaptic membrane	[43,88]

Abbreviations: Akt, protein kinase B; ATF-2, activating transcription factor 2; $[Ca^{2+}]$n, nuclear calcium; CaMK, Ca2+/calmodulin-dependent protein kinase; COS-7, monkey kidney fibroblasts cell line; cTnI, cardiac troponin I; ERK, extracellular signal-regulated kinases; HEK, human embryonic kidney cells; HEK293, human embryonic kidney cells; IL1r1, interleukin 1 receptor, type I; IP3, inositol trisphosphate; NF-κB, factor nuclear kappa B; NOS, nitric oxide synthase; p-, phosphorylated; PDGF, platelet-derived growth factor; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; Ripk2, receptor-interacting serine/threonine-protein kinase 2; SMCs, smooth muscle cells; Tnfrsf1b, tumor necrosis factor receptor superfamily member 1B; VECs, vascular endothelial cells.

have also been described. These messengers influence several processes, including the redox status, transcription, cell proliferation, and tumorigenesis, as summarized in Table 1. However, whether nGPCR transduction mechanisms are similar to those described for mGPCRs needs to be clarified. nGPCRs have been studied with in situ microinjections or using isolated nuclei, allowing the identification of discriminative effects concerning the activation and signaling events triggered by nGPCRs versus mGPCRs. However, most of the methodologies used to study nGPCRs were developed for mGPCR studies (e.g., cell-permeable fluorescent dyes or appropriately targeted biosensors), resulting in limitations to targeting nGPCRs owing to their permeability and ability to discriminate between mGPCR and nGPCRs. Therefore, improved methodologies are required to obtain more discriminative information about the transduction mechanisms associated with nGPCRs.

nGPCRs can be constitutively active or activated by endogenous ligands. Extracellular ligands might reach nGPCRs through cellular uptake via selective transporters (e.g., noradrenaline [36]), through membrane exchangers (e.g., glutamate [37]), or via endocytosis through caveolin-coated pits (e.g., urotensin-II [38]). nGPCR exposure to ligands might differ from that observed for

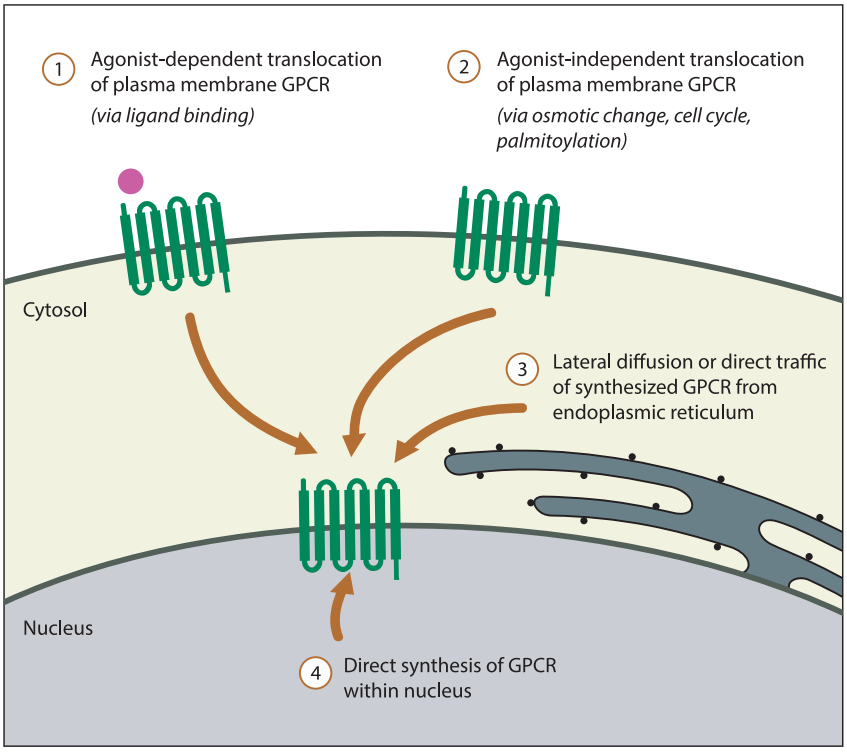

FIGURE 1

Schematic representation of conceivable origins for nuclear full-length G-protein-coupled receptors (GPCRs). Nuclear membranes might be an alternative destination for plasma membrane GPCRs that undergo nuclear translocation via an agonist-dependent (1) and/or -independent (2) pathway. Resident nuclear membranes GPCRs might be synthesized in the endoplasmic reticulum (ER) (3) or within the nucleus (4). (1) Receptor translocation from the cell membrane to the cell nucleus could occur as part of an agonist-mediated internalization event. Binding of an agonist to a receptor induces a first signaling phase at the cell membrane, which, after a long exposure, results in the desensitization and internalization of the receptors, culminating in nuclear translocation (frequently via small GTPases/importins mechanisms). (2) Other events and/or stimuli might also induce receptor internalization and nuclear translocation independently of agonist binding, such as palmitoylation or osmotic changes. (3) Nuclear import of newly rough ER-synthesized receptors can occur via lateral diffusion (given that the outer nuclear membrane is continuous with the ER) or from vesicular transport after ER and/or *trans*-Golgi network post-translational modifications. (4) Protein synthesis has been reported to occur in the nucleus, and is a plausible source of nuclear GPCRs.

mGPCRs because of the differential permeability of the extracellular ligands and of preferentially exposure to intracellular ligands that normally do not reach and influence mGPCRs. Indeed, nGPCRs might be preferentially activated by endogenous ligands that can be synthetized within the cell by enzymes located in the vicinity of the respective nGPCR, ensuring ligand bioavailability in the microenvironment of nuclear receptors. This type of process occurs for various ligands, including lipid mediators, such as prostanoids [39], platelet-activating factor, and lysophosphatidic acid [31], as well as peptides, namely apelin, bradykinin, Ang II [40], or endothelin [41]. All these attributes open new possibilities for determining pharmacological responses among these receptor subpopulations in future studies.

Nuclear GPCRs versus cell membrane GPCRs

The nuclear location of nGPCRs appears to be both cell type and tissue specific. Furthermore, the GPCR subtype has also been suggested as a feature contributing to differential nuclear versus cell membrane location. Different receptor densities have been observed at the cell and nuclear membranes, favoring one or other of the locations [34,42]: the nuclear membrane appears to be the preferential location for some GPCRs (e.g., ET_B [29]) whereas, for others, an alternative destination has been observed (e.g., AT_1 receptor [21]).

nGPCRs and mGPCRs usually present identical structures with a heptahelical chain sequence. However, there are exceptions, where only a small functional portion of the heptahelical chain might be translocated into the nuclear membrane, as reported for the Frizzled-2 (FZD_2) receptor, the C terminus of which is cleaved and then translocated into the nuclear membrane [43]. In some cases, GPCRs might undergo differential post-translational modifications between the cell and nuclear membranes [44]. For instance, the cell surface ET_B receptor undergoes *N*-glycosylation but this is absent in its nuclear counterpart [29].

Activation of nGPCRs can trigger responses aligned with those elicited by their respective cell surface counterparts. In this regard, similar signaling events have been described concerning the nuclear and cell surface mGlu5 receptors. Nuclear mGlu5 receptors have similar roles (i.e., induction of oscillatory Ca^{2+} responses) to those attributed to the cell surface mGlu5 receptors, with identical impacts on synaptic plasticity and the growth and/or differentiation of striatal neurons [45].

Interestingly, differential signaling between nGPCRs and mGPCRs has also been observed, such as in the case of sphingosine

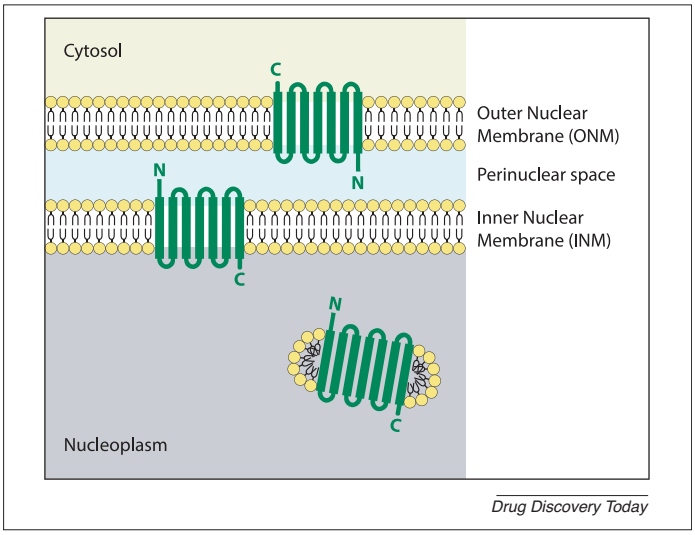

FIGURE 2

Schematic representation of the predicted topology of G-protein-coupled receptors (GPCRs) in the nucleus. Nuclear GPCRs were reported to be located at either nuclear envelope membranes (inner or outer) probably positioned in such a way that signals would be directed to the cytosol or nucleus. In some cases, nuclear GPCRs have been found within the nucleoplasm, possibly at the nuclear envelope invaginations or incorporated in micelles.

1-phosphate (S1P) subtype 1 ($S1P_1$) receptors. In unstimulated T cells, these receptors were not detected in the nucleus, whereas they were found to be involved in cell migration when located in the cell membrane. However, in stimulated T cells, nuclearization of most of the cell surface $S1P_1$ receptors appears to be induced and triggers a decrease in cell proliferation [46]. Another example of discriminated signaling events triggered by nGPCRs and cell surface counterparts is the PAR2 receptor: activation of the cell surface PAR2 receptor leads to expression of genes related to vessel maturation [47], whereas nuclear PAR2 receptor activation stimulates the recruitment of transcription factors that promote angiogenesis [47].

Nuclear GPCRs in pathological conditions

An increasing body of evidence has revealed that nGPCRs are involved in numerous key physiological and/or pathophysiological conditions, predominantly in the cardiovascular and nervous systems, but also in the reproductive system, liver, and kidney (Table 1). The density of nGPCRs also appears to be modified in pathological conditions. For example, nGPCR upregulation has been observed in cancer, pain and heart failure, whereas their downregulation was reported in hypertension. For instance, a higher density of nuclear VPAC1 receptors in high-grade gliomas was reported, possibly constituting a mechanism of tumor resistance [48]; cysteinyl leukotriene 1 ($CysLT_1$) receptors are present in the ONM, after internalization induced by prolonged exposure to leukotriene D4, and are upregulated in cancer [49]; in spinal dorsal horn neurons, ~80% of mGLU5 receptors are intracellularly located, mainly within nucleus (~60%), where they are functional active, and have recently been associated with persistent pain [50]; an increased density of AT_1 receptors was described in a heart failure model, associated with collagen gene expression and secretion and with the regulation of fibroblast proliferation [7]; the nuclear location of PTH1 receptors appears to be favored upon metabolic deprivation, whereas, under standard metabolic conditions, the cell surface and nuclear receptors are both present in similar amounts [25]. By contrast, in hypertension, the expression of nuclear AT_1 appears to be reduced in the kidney, probably as a compensatory mechanism because of increased levels of Ang II [51]. Nevertheless, further studies are needed to clarify the impact of nGPCRs in pathological conditions to evaluate their relevance as putative therapeutic targets.

Interestingly, the expression of nuclear C-X-C chemokine type 4 (CXCR4) receptors has been associated with significantly better outcomes in early-stage non-small cell lung cancer [52], suggesting that some nGPCRs could be used as predictors of disease outcome. It is also conceivable that, after the improvement and validation of methodologies that allow refined discrimination between effects ascribed to different GPCRs populations, nGPCRs could be used to monitor the evolution of disease.

Are nuclear GPCRs pharmacologically targetable?

The existence of GPCRs in the nucleus opens new opportunities and challenges for pharmacological intervention using these targets. GPCRs are targets of approximately one-third of drugs currently used in the clinic, acting on >100 types of receptors, and 94% of them are class A GPCRs [4]. More than 80% of these drugs target GPCRs such as aminergic receptors (adrenoceptors, muscarinic acetylcholine, histamine, dopamine, and 5-hydroxytryptamine receptors) and opioid receptors (gpcrdb.org) signaling mostly via the $G\alpha_{i/o}$ (~38%), $G\alpha_{q/11}$ (~29%), and $G\alpha_s$ (~24%) subunits [53]. Therefore, it is plausible that some of the effects of these drugs result from actions on nGPCRs. For example, activation of α_{1A}-adrenoceptors promotes cell cycle arrest, hypertrophy, and the differentiation of rat-1 fibroblasts into smooth muscle cells as well as the expression of negative cell cycle regulators by a mechanism independent of the cAMP/PKA signaling pathway [54]. Although not addressed, the involvement of nuclear adrenoceptors might explain these effects. Thus, the possibility that nuclear α_{1A}-adrenoceptors contribute to the clinical effects of α_{1A}-adrenoceptor antagonists used in the treatment of benign prostatic hyperplasia cannot be discarded. The same rationale can be applied to $CysLT_1$-selective antagonists, such as montelukast, used in the treatment of asthma, or to AT_1 receptor antagonists, such as losartan, used in hypertension and heart failure, where the reported presence of the respective receptors in the nuclear membranes might partially contribute to their clinical efficacy.

In addition to drugs that interact directly with receptors, several others might influence the intracellular availability of ligands indirectly and, possibly, the ligand concentration in the microenvironment of nuclear receptors. The most obvious example is zileuton (a 5′-lipoxygenase inhibitor used in the treatment of asthma; 5′-lipoxygenase is an enzyme involved in the initial events of leukotriene synthesis, which occurs at the nuclear membrane [55]): when 5′-lipoxygenase is inhibited by zileuton, it impacts the concentration of leukotrienes available for nuclear leukotriene receptors. Other examples of drugs the therapeutic effects of which might have a contribution from nuclear GPCRs include captopril and other inhibitors of angiotensin-converting enzymes. These drugs interfere simultaneously with two endogenous ligands (a decrease in Ang II and the accumulation of bradykinin), the

receptors of which (AT_1 and AT_2 receptors [6,7,22,56,57] and bradykinin B_1 and B_2 receptors [58,59], respectively) were reported to have a nuclear location. In terms of adrenoceptors, several drugs are envisaged to have the potential to interfere with the concentration of their endogenous ligands (noradrenaline and adrenaline), namely monoaminoxidase (MAO) inhibitors, reserpine, and amphetamines. MAO inhibitors have been used in the treatment of depression and Parkinson's disease, and their effects have been explained by sparing monoamines (such as noradrenaline, adrenaline, dopamine, histamine, and serotonin) from metabolism and, consequently, favoring their cytosolic accumulation in the synaptic vesicles. However, such accumulation can also impact the ability of these monoamines to reach other intracellular targets, namely nGPCRs. Therefore, the involvement of nGPCRs could explain the alterations in gene expression caused by MAO inhibitors [60,61]. nGPCRs might also have a role in the effects mediated by reserpine. Reserpine, an antipsychotic drug (also used in hypertension), inhibits the vesicular ATP/Mg^{2+} pump, leading to a cytosolic increase in monoamines that might target nuclear adrenoceptors. This could explain the increase in gene transcription observed after reserpine administration [62,63]. The involvement of nGPCR-mediated effects is also a possibility in the case of recreational drugs with a high risk of addiction, such as amphetamines: the stimulatory effects of amphetamines result from the transient facilitation of neurotransmission, which is caused by monoamine transport from the cell, instead of the usual transport into cell [64]. However, addiction to these drugs is coupled with long-lasting transcriptional alterations that require alternative explanations [65,66]. The possibility that they result from a reduction in the number of messengers available intracellularly to activate nGPCRs is a tempting hypothesis.

The development of new drugs with higher affinity toward nGPCRs than to their cell surface counterparts is a challenging field of research. Therefore, an improved understanding of the different signaling events triggered by nGPCRs and their cell surface counterparts will be relevant to enable the development of drugs targeting solely nGPCRs. Drugs with preferable activity on nGPCR membrane receptors should be designed using techniques such as molecule modification through the addition of a 'caging' moiety, a removable functional group that increases cell permeability and decreases affinity for the cell surface receptor [67]. This type of approach could be sufficient to ensure the activation of nGPCRs, although without discriminative properties that could rule out the activation of mGPCRs on the cell surface. Other approaches could be the use of nanoparticles [68] or cell-penetrating peptides [69] that enter cells usually by endocytosis and/or can be coupled with a NLS peptide, taking advantage of the natural active transport system of the cell into the nucleus. The use of 'Trojan Horses' (i.e., drugs inside a carrier that is sensitive to intracellular enzymes) could be a strategy to circumvent the activation of cell membrane receptors and to deliver drugs preferentially into the intracellular environment, providing more possibilities to be translated into clinical practice.

Concluding remarks

GPCRs were thought to solely convert extracellular stimuli into intracellular responses, while being located exclusively on cell surface. However, over the past few decades, the intracellular location of GPCRs in nuclear membranes was verified, challenging the classic view of GPCRs. nGPCRs might be specifically or additionally activated with overlapping and/or unique signaling events comparative to those of their cell surface counterparts. It has been hypothesized that the final downstream effects attributed to a certain GPCR might result from the integration of extracellular and intracellular signaling pathways.

Studies thus far have led to increased insights into the involvement of nGPCRs and their differential signaling in key physiological and pathophysiological processes. In addition, the density of some nGPCRs is known to be increased in several pathological conditions, highlighting them as putative therapeutic drug targets. Therefore, the nuclear location of GPCRs challenges our traditional understanding of GPCR pharmacology and exponentially increases their complexity. Comprehensive pharmacological studies are urgently needed for the development of new optimized drugs with activity on a desired intracellular response to reach the directed therapeutic outcome. In addition, further studies are required to understand and challenge the classic pharmacological view of drugs used in clinical practice the mechanisms of action of which have been attributed so far solely to GPCRs located in the cell membrane. Thus, our new understanding highlights the complex diversity of GPCR pharmacology, requiring the inclusion of different locations and/or functions for GPCRs in physiopathology and disease.

Conflicts of interest

The authors report no conflicts of interest.

Acknowledgments

M.V. thanks the Portuguese Foundation for Science and Technology (FCT) and PhD Program in Medicines and Pharmaceutical Innovation (i3DU) for the PhD grant PD/BD/135460/2017 funded by the European Social Fund of the European Union and national funds FCT/MCTES. FCT is also acknowledged for grant UID/QUI/50006/2019 with funding from FCT/MCTES through national funds.

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