View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Biochimica et Biophysica Acta 1813 (2011) 1495–1503

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Intracellular α_{2C}-Adrenoceptors: Storage depot, stunted development or signaling domain?

Maqsood A. Chotani [a,b], Nicholas A. Flavahan [c,*]

[a] Center for Cardiovascular and Pulmonary Research, The Research Institute at Nationwide Children's Hospital, Columbus, OH 43205, USA
[b] Department of Pediatrics, The Ohio State University, Columbus OH 43210, USA
[c] Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University, Baltimore MD 21205, USA

ARTICLE INFO

Article history:
Received 16 March 2011
Received in revised form 3 May 2011
Accepted 6 May 2011
Available online 14 May 2011

Keywords:
Signal transduction
Endoplasmic Reticulum
Golgi
Cold

ABSTRACT

G-protein coupled receptors (GPCRs) are generally considered to function as cell surface signaling structures that respond to extracellular mediators, many of which do not readily access the cell's interior. Indeed, most GPCRs are preferentially targeted to the plasma membrane. However, some receptors, including α_{2C}-Adrenoceptors, challenge conventional concepts of GPCR activity by being preferentially retained and localized within intracellular organelles. This review will address the issues associated with this unusual GPCR localization and discuss whether it represents a novel sub-cellular niche for GPCR signaling, whether these receptors are being stored for rapid deployment to the cell surface, or whether they represent immature or incomplete receptor systems.

© 2011 Elsevier B.V. All rights reserved.

To function correctly within a complex environment, cells must detect signals in the extracellular space and initiate appropriate intracellular responses to address the challenges imposed by the stimulus. G-protein coupled receptors (GPCRs) represent the largest family of proteins that enable cells to respond to signals that are generally restricted to the extracellular milieu. They can mediate the effects of ions, hormones, neurotransmitters, local autocoids, and extracellular matrix components. GPCR activation is generally mediated by binding of signals to the extracellular component of the receptor, which cause conformational changes in the receptor protein and lead to activation of heterotrimeric G-proteins located at the inner surface of the plasma membrane. G-proteins then initiate changes in cellular function by regulating the activity of intracellular signaling mediators including enzymes, ion channels and transcription complexes. Despite our standard view of GPCRs as cell surface recognition sites, there is increasing evidence of a physiological role for intracellular GPCRs. Although intracellular receptors can represent a novel aspect of cell surface GPCR signaling following their internalization, GPCRs can also be captured and retained within the biosynthetic pathway before reaching the cell surface. The present review will address the interesting dilemma resulting from this preferential localization, focusing particular attention on the α_{2C}-adrenoceptor (α_{2C}-ARs), and whether these intracellular receptors represent a novel sub-cellular niche for GPCR signaling, whether these receptors are being stored for rapid deployment to the cell surface, or whether they represent immature or incomplete receptor systems.

1. Physiological role of α_{2C}-Adrenoceptors

α_{2C}-Adrenoceptors (α_{2C}-ARs) belong to the α_2 sub-family of adrenoceptors that include α_{2A} and α_{2B}-subtypes. α_{2C}-ARs are 462 amino acid long, class A GPCRs that are encoded by an independent intronless gene located on human chromosome 4p16 [1–3]. The physiological role of these receptors is tissue and cell-type dependent, and has been primarily elucidated by animal models following genetic ablation or overexpression, by identification and characterization of polymorphisms in the human population, and by examination of cells expressing endogenous receptors. For example, the central and sympathetic nervous system express α_{2C}-ARs, which plays a key role in pre-synaptic feedback inhibition (autoinhibition) of neurotransmitter release. Prejunctional α_{2A}-ARs regulate norepinephrine release during high-frequency neuronal stimulation and likely represent a mechanism for coordinating intense acute sympathetic responses. In contrast, α_{2C}-ARs modulate norepinephrine release at low frequency stimulation and may primarily regulate basal long term neurotransmitter release [4,5]. The physiological significance of α_2-AR-mediated autoinhibition was appreciated in mice with single or double knockout of α_{2A}- and α_{2C}-AR subtypes, and by the discovery of a variant with four amino acids deletion in the third intracellular loop of α_{2C}-AR subtype (α_{2C}-Del322-325) in the human population, which impairs effector coupling [5–7]. Mice deficient in both α_{2A}- and α_{2C}-ARs (double knockout) show elevated circulating plasma norepinephrine

* Corresponding author at: Department of Anesthesiology, Johns Hopkins University, Ross Research Building, R1159, 720 Rutland Ave, Baltimore, MD 21205, USA. Tel.: +1 410 502 6738; fax: +1 410 502 7007.
E-mail address: nflavah1@jhmi.edu (N.A. Flavahan).

0167-4889/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2011.05.006

concentrations, and develop cardiac hypertrophy and dysfunction under non-stressful conditions. When challenged with chronic cardiac left ventricular pressure overload and stress using transverse aortic constriction, mice with deletion of α_{2A}-ARs or α_{2C}-ARs (single knockouts) are also susceptible to increased incidence of hypertrophy and fibrosis, heart failure, and mortality compared with wild-type and α_{2B}-AR-deficient mice [6]. Similarly, individuals with α_{2C}-Del322-325 are at increased risk of developing heart disease [8,9], and heart failure patients with this variant have adverse clinical status with decreased cardiac function [6]. Studies in mice have also shown that central nervous system α_{2C}-ARs have a discrete role in behavioral responses that include startle reflex, stress response, and locomotor activity [10], while the α_{2A}-AR subtype is responsible for sedation, analgesia, and anesthetic-sparing effects [11,12].

Within the vasculature, α_2-ARs are prominently expressed on contractile vascular smooth muscle cells (VSM) in the blood vessel wall [13]. The constrictor activity of α_2-ARs is inversely related to the size of the blood vessel and is most prominent in small vessels. For example, VSM α_2-ARs mediate constriction of arterioles (resistance vessels) and veins, but not large arteries [14–16]. These arterioles serve the unique role of "vascular faucets", determining blood flow to organs and the cutaneous circulation and in generating peripheral resistance [15]. α_2-ARs, therefore, play an essential physiological role in modulating blood vessel diameter and flow. The remarkable heterogeneity in α_2-AR activity at different vascular sites is due to differences in activation of α_2-AR gene promoters [16]. Analysis of the intact cardiovascular system in mice with genetic ablation of α_2-AR subtypes suggested that α_{2A}- and α_{2B}-ARs mediated vasoconstriction to α_2-AR stimulation, with α_{2A}-ARs mediating the central antihypertensive response [17–19]. α_{2C}-ARs were not considered to be involved in vascular regulation, and were thought to be vestigial or silent receptors [12,17,18]. Indeed, in small cutaneous arteries of the mouse tail, α_2-AR constriction at 37 °C is mediated by α_{2A}-ARs, with no apparent role for α_{2C}-ARs [20]. However, moderate cooling (to 28 °C) dramatically and rapidly increases the functional activity of α_{2C}-ARs, enabling these receptors to contribute to physiological cold-induced augmentation of cutaneous vasoconstriction [20]. Cold-induced cutaneous vasoconstriction plays a protective physiological role, reducing loss of body heat and enabling maintenance of a normal body core temperature [21]. In vivo studies have confirmed the role of α_{2C}-ARs in cooling-triggered vasoconstriction in mouse and human skin [22,23]. In human cutaneous arteriolar VSMs, endogenous α_{2C}-AR expression is markedly increased in response to serum stimulation and by elevations in intracellular cyclic AMP levels, which can result from increased expression of cyclooxygenase-2 [16]. α_{2C}-AR expression was mediated by activation of the cyclic AMP-responsive EPAC (exchange proteins activated by cyclic AMP) and the Ras-family GTPase Rap1, which caused transcriptional activation of the α_{2C}-AR gene through JNK/c-Jun signaling [24,25]. Protein kinase A, the classic cyclic AMP signaling pathway, did not have a physiological role in α_{2C}-AR expression. Together, these studies point to local action and regulation of α_{2C}-ARs in the microcirculation, and supported the physiological role of α_{2C}-ARs as "stress-receptors" of the vascular sympathetic system [16,20,24,26].

2. α_{2C}-Adrenoceptors as intracellular receptors

In contrast to α_{2A} or α_{2B}-ARs, α_{2C}-ARs have a prominent intracellular localization when expressed endogenously [27–29] or in reconstituted cell systems [4,28,30–35]. By introducing a thrombin-cleavable N-terminus into α_{2C}-ARs, Daunt et al. demonstrated that intracellular α_{2C}-ARs were not cleaved by thrombin, suggesting that they represented a resident and non-cycling pool of receptors (at least in NRK cells) [31]. The magnitude of this intracellular pool of α_{2C}-ARs and the relative ability of these receptors to access the cell surface is dependent on the cell type, with the largest proportion of cell surface receptors occurring in neuroendocrine cell lines (AtT20, PC12 cells), although these cells still have a significant intracellular component [30,33]. It has generally been assumed that the mechanisms responsible for intracellular retention of α_{2C}-ARs are similar between different cell types. However, available evidence suggests that it is not the case and that α_{2C}-ARs are characterized by a number of distinct molecular species and distinct subcellular localizations, which is determined by the cell type and experimental conditions.

α_{2C}-ARs have generally been identified as three molecular species with approximate molecular weights of 50–55 kD, 70–80 kD and 100–120 kD [4,30,33,34,36]. The extracellular N-terminus of α_{2C}-ARs is N-glycosylated at two sites, and all three receptor species are glycoproteins. The 50–55 kD species appear to represent a core glycosylated protein, the 70–80 kD species a more extensively glycosylated receptor, and the 100–120 kD species comprising an SDS-resistant dimer of the 50–55 kD protein [4,30,33,34]. After mutation of the N-glycosylation sites (N19 and N33) or treatment with peptide:N-glycosidase F (PNGaseF), which cleaves N-linked oligosaccharides, the 50–55 kD and 70–80 kD forms are reduced to a ~46 kD protein, and the 100–120 kD dimer reduced to ~90 kD species [30,33, authors unpublished observations]. Movement of glycoproteins through the synthetic pathway can be assessed using endoglycosidase H (EndoH), which distinguishes between immature, high-mannose oligosaccharides (sensitive) and complex, mature moieties (insensitive). Mannosidase II (MannII) completes the mannose trimming reactions and gives rise to EndoH-resistant glycoproteins [37]. MannII is most often located in medial and trans-Golgi compartments, although its exact location appears to be cell type specific [37,38]. The 50–55 kD and 100–120 kD species are EndoH-sensitive [30,33], which suggests that they represent immature forms of the receptor and have not accessed the distal components of the Golgi compartments. In contrast, the 70–80 kD species is resistant to EndoH [30,33] suggesting that these α_{2C}-ARs have progressed to the medial and transGolgi compartments. Indeed, when the cell-surface component of α_{2C}-ARs were selectively analyzed using cell membrane fractionation (HEK 293 cells), extracellular immunoprecipitation (HEK 293), or biotinylation followed by photoaffinity labeling (MDCK II cells), the α_{2C}-AR species on the cell surface was found to be exclusively the 70–80 kD molecular species [35,39]. Similarly, in cell types where α_{2C}-ARs have a predominant cell surface expression (AtT20, PC12) there is significant expression of this EndoH-resistant 70–80 kD species, whereas in cell types that have a predominant or exclusive expression of intracellular α_{2C}-ARs (e.g. COS7 cells) expression appears to be restricted to the Endo H-sensitive forms [33]. These results are consistent with the 70–80 kD α_{2C}-AR species representing a mature form of the receptor and the other species representing immature components that have not progressed to the distal Golgi compartments.

Based on co-localization with organelle marker proteins or by purification of subcellular membranes, intracellular α_{2C}-ARs have been proposed to be localized and retained within the endoplasmic reticulum (ER), cis/medial Golgi and/or trans-Golgi compartments. In Rat1, NRK and HEK293 cells, Hurt and colleagues observed that staining for α_{2C}-ARs overlapped with BiP or calreticulin (markers for ER) and with giantin or Mann II (used as markers of cis/medial Golgi), but lacked significant overlap with a trans-Golgi/endosome marker (mannose 6-phosphate receptor, M6PR) [30,31,33]. In a more recent study in HEK 293 T cells, Fillipeanu et al. observed that α_{2C}-ARs in HEK 293 T cells were co-localized almost exclusively with pDsRed2-ER, an ER marker, but only "occasionally" with GM130, a marker for the cis-Golgi [32]. Despite slight differences in localization, these investigators came to divergent conclusions regarding the retention of α_{2C}-ARs. Fillipeanu et al. proposed that intracellular retention of α_{2C}-ARs resulted from an intrinsic defect in folding and export of the receptor from the ER [32]. The quality control system of the ER is known to capture genetic mutations in certain proteins (e.g. CFTR ΔF508, deletion of phenylalanine at residue 508), which result in improper folding of the molecules [40–43]. Indeed, prolonged treatment (18 h) of HEK 293 T cells with chemical chaperones

(DMSO, glycerol), which correct ER retention of genetic mutations [40–42], increased the proportion of α_{2C}-ARs able to bind the radioligand RX821002 [32]. Misfolded α_{2C}-ARs in the ER do not effectively bind this radioligand [33], so the observed increases in RX821002 binding would be consistent with correction of a folding defect [32]. In contrast to this proposal, Hurt and colleagues concluded that intracellular α_{2C}-ARs are not misfolded but are retained in the ER by a distinct mechanism [30,33]. Indeed, they demonstrated using density-purified membrane preparations (or crude membrane preparations) that α_{2C}-ARs within the ER could bind RX821002 [30,33]. They subsequently identified a novel ER luminal retention motif (ALAAALAAAAA) contained in the 'extracellular' amino-terminus of the α_{2C}-ARs [30]. Deletion or mutation of this retention motif was associated with increased maturation of α_{2C}-ARs and increased localization of the receptors on the cell surface. Wild-type (wt) α_{2C}-ARs were expressed almost exclusively as immature EndoH-sensitive ~50 kD and ~100 kD species in HEK 293 cells [30]. However, mutation or deletion of the putative ER luminal retention motif enabled expression of a prominent EndoH-resistant ~70 kD α_{2C}-ARs species, which is consistent with progression of the immature species through the distal compartments of the Golgi [30]. The divergent conclusions of these studies are not mutually exclusive. Indeed, these studies may have focused on distinct populations of ER-retained α_{2C}-ARs. Identification of a novel ER retention motif in correctly-folded α_{2C}-ARs does not negate the possibility that some α_{2C}-ARs are also retained as misfolded receptors and can be rescued by chemical chaperones. In contrast to Hurt and colleagues [30,33], Fillipeanu et al. [32] focused on the emergence of new RX821002 binding sites, which could have selectively tracked the processing and maturation of misfolded α_{2C}-ARs. However, an increase in RX821002 binding sites (when employing crude membrane preparations or intact cells [32,44,45]) need not be an indication of cell surface receptors (see Section 3.2). Therefore, the chaperone-induced appearance of new RX821002 binding sites observed by Fillipeanu et al. [32] could still be retained in the ER (via the ER luminal retention motif) or other intracellular location [33,46]. Hurt and colleagues [30,33] focused on alternate approaches to study receptor maturation and localization (e.g. immunoblot, immunofluorescence), which would be expected to identify misfolded and correctly-folded α_{2C}-ARs. Indeed, deletion or mutation of their novel ER retention motif did not cause complete maturation of α_{2C}-ARs, which could reflect the continued presence of misfolded receptors [30]. Finally, Hurt and colleagues [30,33] and Fillipeanu et al. [32] could potentially be studying the same population of ER-retained α_{2C}-ARs, with the ER luminal retention signal maintaining the receptors in a non-functional conformation with reduced ability to bind RX821002. This is considered unlikely because removal of the ER retention motif (replaced with the α_{2A}-AR N-terminus) did not appear to increase the density of RX821002 binding sites [30].

The cytoplasmic C-terminus of the α_{2C}-AR contains an RRRRR motif (residues 454 to 458), which has been demonstrated to be a powerful ER retention and retrieval mechanism. Indeed, when inserted into the C-terminal of other plasma membrane proteins, this α_{2C}-AR motif causes them to be retained or retrieved to the ER and prevents their cell surface expression [47,48]. This motif is an unusual form of the RXR family of ER retention and retrieval system, which interacts with the coatamer protein complex I (COPI) and mediate retrograde transport of proteins from the proximal region of the Golgi back to the ER [42,47–49]. The RRRRR motif is also present on the cytoplasmic tail of KA2 kainate receptors, which causes the receptors to be retained in the ER in a COPI-dependent manner in COS7 or HEK 293 cells [49]. Mutation of the RRRRR motif decreased the association of KA2 with the COPI-subunits, β-COP and α-COP, and caused a dramatic increase in the plasma membrane levels of the protein [49]. Therefore, for α_{2C}-ARs to escape the ER and proximal Golgi compartments, this powerful retention signal must be shielded or countered. For example, interaction with 14–3–3 protein family members can prevent COPI binding to RXR motifs and enables protein release from this ER retrieval pathway [49,50]. In Rat1 cells, where α_{2C}-ARs have a prominent co-localization with the resident ER protein calreticulin, removal of the RRRRR motif was reported not to alter the intracellular localization of the receptor [30], although the identity of the intracellular compartment for the mutant receptors was not defined. The presence of an additional ER luminal retention motif in the extracellular region of α_{2C}-ARs (as described above) [30] would contribute to continued ER retention of α_{2C}-ARs even in the absence of the RRRRR motif. However, deletion or mutation of this ER luminal retention motif enabled significant maturation and translocation of α_{2C}-ARs to the cell surface [30], which suggests that the RRRRR motif was not functioning as an ER retention or retrieval signal. It is not clear if this reflects interaction of α_{2C}-ARs with counteracting signaling mediators (e.g. 14–3–3) or whether correctly-folded α_{2C}-ARs (but perhaps not misfolded receptors) normally mask the RRRRR motif [49,50].

In addition to retention of immature α_{2C}-AR species in the ER and proximal Golgi compartments, mature α_{2C}-ARs have also been reported to be retained in the trans-Golgi compartment. In HEK 293 cells, Jeyaraj et al. observed co-localization of α_{2C}-ARs in the trans-Golgi compartment (ECFG-tagged peptide from the trans-Golgi enzyme β 1,4-galactosyltransferase), and the sole expression of a mature EndoH-resistant ~70 kD α_{2C}-ARs species in density-purified Golgi membranes [39]. In PC12 cells, which have a prominent cell surface population of α_{2C}-ARs [33] and a variable population of intracellular receptors [33,51], there was considerable overlap between the intracellular pool of α_{2C}-ARs and mannose 6-phosphate receptor (M6PR), which is a marker for trans-Golgi and endosomes [33]. Intracellular α_{2C}-ARs in MDCK II cells were also considered to be predominantly in the trans-Golgi compartment [35], which was based on the partial co-localization of α_{2C}-ARs with Mann II. As discussed above, this enzyme is most often localized to the medial and trans-Golgi compartments, but its localization is cell type specific and its co-localization with α_{2C}-ARs has often been used as evidence for cis/medial-Golgi localization. The exact localization of α_{2C}-AR within the Golgi therefore cannot be clearly defined based on Mann II. However, co-localization with Mann II is associated with a higher expression of the mature 70–80 kD α_{2C}-AR species and a higher level of cell surface receptors compared to cell types where the predominant α_{2C}-AR localization is in the ER [31,33,35]. Therefore, colocalization with Mann II indicates that α_{2C}-ARs have accessed the distal compartments of the Golgi and are fully mature receptors. Although intracellular α_{2C}-ARs appear to represent a stable pool of resident receptors derived from de novo protein synthesis [31], there is also evidence that cell surface α_{2C}-ARs may be internalized to the trans-Golgi compartment. Indeed, the cell surface population of α_{2C}-ARs in NRK cells were internalized following stimulation with norepinephrine and acquired a perinuclear localization that overlapped with staining for the trans-Golgi/endosome marker M6PR [31]. The apparent trans-Golgi staining for α_{2C}-ARs in PC12 cells may reflect a naïve receptor population or receptors that have been internalized from the cell surface [33]. PC12 cells can synthesize norepinephrine. Although the authors reported that an α_2-AR antagonist did not affect plasma membrane microdomains of α_{2C}-AR, they did not report whether it altered the perinuclear pool of α_{2C}-ARs [33].

Despite the compelling evidence for the presence and retention of α_{2C}-ARs in distal Golgi compartments (including HEK 293 cells), when characterizing a novel ER retention motif Angelotti and colleagues concluded that the "Golgi apparatus would be less likely for the site of action for the α_{2C}-AR trafficking signal in HEK 293 cells because this compartment does not contain many quality control enzymes or chaperones" [30]. Following deletion or mutation of the ER luminal retention motif, approximately 50% of α_{2C}-ARs remained at intracellular sites although there appeared to be significant displacement from the ER marker protein calreticulin [30]. Therefore, Angelotti et al. cannot discount the possibility that once freed from the ER retention system, a significant portion of α_{2C}-ARs may be retained within distal compartments of the Golgi. However, deletion or mutation of the ER luminal retention motif may also have modified

the activity of a trans-Golgi retention signal. In contrast to the statement by Angelotti and colleagues, numerous proteins (including GPCRs, see Section 3.1) are selectively targeted or retained within the distal-Golgi compartments including the trans-Golgi [52–58].

Because of the presence of powerful retention systems for α_{2C}-ARs, overexpression of the receptor might be expected to saturate retention systems and allow α_{2C}-ARs to gain access to the cell surface. Indeed, α_{2C}-AR responses to extracellular agonists are observed in most reconstituted cell systems even those with predominant intracellular localization of the receptor, albeit at a lower level of activity compared to other α_2-AR subtypes. However, in NRK cells, where α_{2C}-ARs have a predominant intracellular localization and only faint plasma membrane staining, this pattern of localization was observed regardless of the level of receptor expression [33]. This suggests that α_{2C}-ARs are not only actively captured by retention systems within the cell, but that they are also actively exported to the cell surface in a manner that bypasses or inhibits the retention signals.

The variation in subcellular distribution and maturation of α_{2C}-ARs between different cell types, and within the same cell type in different studies (e.g. HEK 293 cells [30–32,39,59,60]) suggests that the subcellular trafficking and intracellular retention of α_{2C}-ARs is controlled by signaling pathways that promote or counter the retention and export domains contained within the α_{2C}-AR sequence. Although some of these domains have been identified and characterized, other important regulatory domains and the signaling pathways that control them await identification. The variation in receptor maturation and localization between different studies of the same cell type likely reflect alterations in experimental approach (e.g. serum-containing [39] versus serum-free conditions [32]) that may dramatically impact these regulatory signaling pathways.

3. The Functional significance of intracellular GPCRs and α_{2C}-Adrenoceptors

3.1. Storage depots

Mature GPCRs can be stored in distal Golgi compartments, including the trans-Golgi, and following the appropriate cell stimulation they can be translocated rapidly to the cell surface. For example, the ability of cells to maintain sensitivity to thrombin is mediated in part by translocation of naïve receptors from a stable pool retained in the trans-Golgi compartment [61]. Thrombin (and other protease-activated) GPCRs are activated by a unique mechanism, whereby thrombin cleaves the receptor's amino-terminal exodomain to uncover a tethered peptide ligand [61]. This irreversible proteolytic activation renders the receptor unresponsive to subsequent thrombin-induced activation [61]. Indeed, following stimulation, the receptors are rapidly internalized and targeted to lysosomes for degradation and are not recycled to the cell surface as occurs with other GPCRs (e.g. β2-ARs) [61]. In transfected Rat1 fibroblasts, thrombin receptors localized to the plasma membrane and to a perinuclear distribution, which colocalized with Mann II and the fluorescent ceramide analog C6-NBD-ceramide, markers for the trans-Golgi compartment [62,63]. Thrombin stimulation caused internalization and subsequent degradation of cell surface receptors, which was paralleled by mobilization of these naïve intracellular receptors to the cell surface with almost complete recovery of the cell surface receptor pool and responsiveness to thrombin, together with loss of intracellular receptor storage, all within 1 h [61]. Mobilization of receptors from intracellular pools within the Golgi compartment can also contribute to maintained sensitivity or reduced desensitization of non-protease GPCRs. In HEK 293 cells, M2 receptors were localized to the cell surface and a prominent perinuclear compartment that colocalized with medial/trans-Golgi markers (Mann II, α2,6-sialyltransferase) [64]. The muscarinic agonist carbachol caused a rapid mobilization of this intracellular receptor pool, increasing the density of cell surface receptors while depleting the intracellular compartment [64]. This mobilization of receptors partially masked the agonist-induced internalization of M2Rs associated with receptor desensitization [64]. Activation of cell surface GPCRs can also cause mobilization of distinct cell surface receptors from the trans-Golgi compartment. In human umbilical vein endothelial cells (HUVECs), the non-GPCR vascular growth factor receptor-2 (VEGFR2) is localized to the plasma membrane whereas the non-GPCR VEGFR1 has a predominant stable perinuclear localization and co-localizes with the trans-Golgi markers TGN46 and GalT, and also with cis/medial Golgi markers (GM130, Mann II)[65]. Indeed, there appeared to be two distinct pools of VEGFR1: one in the trans-Golgi and one in cis-Golgi, with serum starvation or inhibition of protein synthesis depleting the cis-Golgi pool and increasing the trans-Golgi pool [65]. Activation of HUVECs by VEGF-A, histamine or thrombin caused a rapid mobilization of VEGFR1 from the trans-Golgi to the plasma membrane, attaining a 2-fold increase within 15 min [65]. VEGFR1 mobilization to the cell surface was mediated by elevation in cytoplasmic calcium, was inhibited by the calcium ion chelator BAPTA-AM and stimulated by calcium ionophore (A23187) or thapsigargin, which causes calcium release from intracellular stores [65]. Elevation in cytoplasmic calcium may also contribute to the depolarization-induced translocation of δ opiod receptors (DORs) from intracellular membranes to the cell surface of cerebral and spinal cord neurons [66]. When expressed in PC12 cells, DOR was expressed predominantly at the plasma membrane, which is also where they localize in non-neuronal cells [66]. However, exposure of the cells to nerve growth factor (NGF), which stimulates differentiation of PC12 cells, or to epidermal growth factor (EGF) caused the generation of an intracellular pool of DOR [66]. This was not the result of receptor internalization, and indeed NGF did not stimulate DOR internalization. Rather NGF caused selective intracellular targeting of recently synthesized, mature EndoH-resistant DORs from the biosynthetic pathway [66]. As with VEGFR1 receptors, there was pronounced overlap between DOR staining and that of TGN38 a trans-Golgi marker and with GM130, which is associated with cis-Golgi membranes. Chase experiments confirmed that this intracellular pool is not simply a reflection of increased DOR synthesis and represents a stable pool of intracellular receptors. An undefined Golgi-retention motif was localized to the C terminal of DOR. The intracellular pool of DORs could be rapidly mobilized in response to depolarization with KCl (30 min), which caused decreased amounts of DOR at intracellular sites and increased density at the plasma membrane consistent with translocation of the receptor [66]. Thus, mature intracellular receptors can be rapidly mobilized from the distal compartments of the Golgi compartments to maintain responsiveness or modify the signaling responses to receptor agonists or to uncover responses to independent agonists. In an analogous fashion to receptor desensitization [67], sensitization or resensitization of receptors may therefore occur in a homologous (agonist or receptor-specific) or heterologous (receptor-independent) manner.

In the vascular system, α_2-AR activity is dramatically increased in cutaneous blood vessels, where these receptors play an important role in thermoregulation [14,68–70]. During local or whole body cooling, blood flow to the skin is reduced to prevent heat loss, which is mediated by a reflex increase in sympathetic tone and by a direct effect of cold on the cutaneous blood vessels [21]. This latter effect dramatically and selectively sensitizes the vessels to vasoconstriction mediated by the sympathetic neurotransmitter, norepinephrine, and is mediated by a rapid and selective, cold-induced increase in α_2-AR function [14,68,71–74]. Indeed, in individuals with Raynaud's phenomenon, which is characterized by enhanced sensitivity to the direct effect of cold, α_2-AR antagonism prevents cold-induced vasospastic attacks [75,76]. Cold-induced amplification of α_2-AR activity in cutaneous arteries was subsequently demonstrated to be mediated by a selective effect of cooling to amplify or uncover responses to α_{2C}-

AR activation [20,22]. Indeed, at 37 °C, the constrictor response to α_2-AR stimulation in mouse isolated tail arteries was mediated by α_{2A}-ARs with no apparent contribution from α_{2C}-ARs [20]. However, during cold exposure (28 °C, 30 min), the augmented α_2-AR response was mediated by α_{2C}-ARs and was abolished by inhibition of α_{2C}-ARs [20]. In HEK293 cells transfected with α_2-ARs, α_{2A}-ARs were expressed on the cell surface and responded to activation by regulating adenylyl cyclase activity [39]. Cooling (to 28 °C) did not influence α_{2A}-AR location or function. In contrast, α_{2C}-ARs were not functional at 37 °C and were localized, by sub-cellular fractionation or immunofluorescent techniques, to the trans-Golgi compartment [39]. Cooling (to 28 °C) caused redistribution of α_{2C}-ARs to the cell surface and rescued the α_{2C}-AR functional response, demonstrated by agonist-dependent regulation of adenylyl cyclase [39]. Subcellular fractionation revealed that the α_{2C}-AR leaving the Golgi and translocating to the plasma membrane in response to cold was the mature Endo-H resistant ~70 kD species [39]. This translocation was relatively rapid, enabling a 3 to 4-fold increase in cell surface α_{2C}-ARs within 1 h, and occurred in the absence of new protein synthesis or changes in the total expression of the receptor [39,59,60]. The thermosensor responsible for initiating this effect appears to the mitochondria. On exposure of vascular and other cells to lower temperatures, there was an immediate increase in the generation of reactive oxygen species (ROS) from the mitochondria [59]. Mitochondria-derived ROS activated RhoA and Rho kinase (ROCK), and this signaling pathway was responsible for cold-induced amplification of α_2-AR constriction, for uncovering functional responses to α_{2C}-AR stimulation and for enabling the rapid cold-induced translocation of α_{2C}-ARs to the cell surface [59,60]. Therefore, although originally considered as thermosensors [20,68], α_{2C}-ARs do not respond directly to changes in temperature and are actually thermo-effectors, responding to a cold-induced signal transduction pathway. Therefore, these results indicate that cold-induced vasoconstriction in cutaneous arteries results from a rapid cold-induced functional rescue of silent α_{2C}-ARs, mediated by a cold-induced increase in mitochondrial ROS and activation of RhoA and ROCK. In a reconstituted cell system (HEK293 cells), cold exposure also caused a rapid functional rescue of α_{2C}-ARs, which was paralleled by a ROS and ROCK-dependent rapid translocation of mature α_{2C}-ARs from the trans-Golgi compartment to the cell surface. The mechanisms underlying the ability of RhoA and ROCK to stimulate translocation of α_{2C}-ARs from the trans-Golgi to the cell surface of HEK 293 cells has not been defined [60]. It may reflect actions of ROCK on α_{2C}-ARs, the biosynthetic pathway including chaperones or cargo proteins, and/or changes in plasma membrane microdomains that increase the ability of α_{2C}-ARs to be inserted or remain at the cell surface. Translocation of other GPCRs from the trans-Golgi to the cell surface occurs in response to elevations in intracellular calcium [65,66]. Indeed, calcium and ROCK may be acting through a common signaling pathway, e.g. to regulate myosin-light chain phosphorylation and the cytoskeleton.

Because signaling through ROS/RhoA/ROCK can stimulate α_{2C}-ARs translocation to the cell surface and uncover α_{2C}-AR functional responses [60], α_{2C}-AR activity may be increased by heterologous sensitization, i.e. in response to activation by other vasoconstrictor stimuli. Vasoconstriction to α_2-AR activation is a relatively weak response and is generally rather difficult to observe and analyze [20,77–79]. Indeed, to assess vascular α_2-AR activity, many investigators first activate arteries with an independent constrictor agent (e.g. angiotensin II or serotonin) to help uncover or amplify the α_2-AR response [80–82]. The mechanisms (including the α_2-AR subtype) underlying this "rescue" of α_2-AR function has not been clearly defined, but may reflect mobilization of α_{2C}-ARs, e.g. through activation of RhoA/ROCK. Indeed, in the rat tail artery, which has only a weak α_2-AR constrictor response [73,82], responses to α_2-AR activation were mediated by α_{2C}-ARs in the presence of a pre-constricting agent (serotonin) [83] but not when studied under basal conditions [84].

3.2. Stunted development

Proteins can be captured or retained within the early stages of the biosynthetic pathway as a result of inherent defects in protein folding and maturation, which commonly occur as a result of genetic mutations [e.g. cystic fibrosis transmembrane conductance regulator (CFTR), the ATP-binding cassette subfamily B member 4 (ABCA4), and vasopressin type 2 receptors (V2Rs)], resulting in chaperone-linked retention of these proteins by the quality control mechanisms of the ER [41,42,85,86]. Chaperone-linked capture and retention of normal proteins can also optimize association with key signaling partners, including oligomerization of GPCRs [87–89]. There is evidence that these mechanisms can contribute to the retention of α_{2C}-ARs within the ER.

From the perspective of α_{2C}-ARs and their role in thermoregulation, it is especially intriguing that prolonged exposure of cells to low temperatures counters the ER retention of misfolded mutant proteins including genetic mutations in CFTR, ABCA4 and V2Rs [41,42,85,86]. A genetic deletion of phenylalanine at residue 508 (ΔF508) of the CFTR results in a folding defect in the protein that largely prevents the protein from exiting the ER where it is retained as an immature EndoH-sensitive glycoprotein [41,85]. At low temperatures (27 to 32 °C), ΔF508 CFTR exits the ER, acquires Golgi-specific N-linked glycan modifications rendering it EndoH-resistant, and translocates to the cell surface where it can function as an ion channel [41,85] This is a slow process, being evident only after 12 h and maximal after 72 h [85]. Cold exposure alters the interaction of the mutant CFTR protein with ER chaperones and export proteins: it reduced the interaction between ΔF508 CFTR and HSP90, but increased the association of the CFTR mutant with HSP70 and subsequently with the COPII ER cargo selection subunit Sec24 [41]. The COPII system is responsible for generating transport vesicles for delivery of cargo to the Golgi [90]. Directly disrupting the interaction between ΔF508 CFTR mutant and HSP90 (or co-chaperones) at warm temperatures was also associated with functional and spatial rescue of the mutant protein [90,91]. Exogenous chemical chaperones (e.g. DMSO) can also stabilize the protein fold of mutant proteins, and they promoted ΔF508 CFTR export at physiological temperatures, albeit after prolonged incubation (up to 2 days) [40,41]. Therefore, ΔF508 CFTR appears to be trapped in a chaperone-linked folding intermediate within the ER, which can be countered by prolonged exposure to low temperatures. Interestingly, this cold-induced rescue of ΔF508 CFTR and V2R is cell-type specific indicating that appropriate signaling/chaperone systems need to be in place [41,42].

The cold-induced, slow functional and spatial rescue of misfolded immature mutant proteins from the ER is clearly very different from the reported rapid cold-induced translocation of mature α_{2C}-ARs from the trans-Golgi to the cell surface. However, Filipeanu and colleagues recently concluded that the intracellular retention of α_{2C}-ARs in HEK 293 T cells resulted from an intrinsic defect in folding and export of the native receptor from the ER [32]. Furthermore, they proposed that exposure to low temperatures facilitates receptor stabilization and allows its inclusion in the export trafficking pathway [32]. Exposure of HEK 293 T cells to cool temperatures (30 °C) caused a slow increase in the density of α_{2C}-ARs that bound the α_2-AR ligand RX821002, which attained statistical significance after 6 h and peaked after 18 h [32]. Similarly, prolonged treatment (18 h) with chemical chaperones (DMSO, glycerol) or HSP90 inhibitors also increased RX821002 binding density at 37 °C but had no effect at 30 °C [32]. As with the ΔF508 CFTR mutant protein, co-immunoprecipitation experiments demonstrated interaction between HSP90 and α_{2C}-ARs at 37 °C, which was dramatically reduced following prolonged exposure to 30 °C (18 h) [32]. These effects are consistent with observations on misfolded mutant proteins, but are in marked contrast to previous reports demonstrating a rapid cold-induced translocation of mature α_{2C}-ARs from the trans-Golgi to the cell surface [39,59,60]. In contrast

to those previous studies, which relied on antibody-based techniques to assess α_{2C}-AR localization [39,59,60], Filipeanu and colleagues employed radioligand binding of RX821002 to crude cell membrane preparations or intact cells in an attempt to quantify cell surface α_{2C}-ARs [32,44]. Crude cell membrane preparations contain receptors from plasma membranes and intracellular organelles and cannot be used to quantify cell surface receptors. Indeed, RX821002 can bind intracellular α_{2C}-ARs in NRK cells when present within crude membrane preparations or density-purified intracellular membranes [33]. Likewise, when used in intact cells, RX821002 can bind to cell surface and intracellular α_{2C}-ARs [46]. Incorrectly processed, misfolded α_{2C}-ARs cannot effectively bind RX821002 [30]. Therefore, focusing on increased RX821002 binding sites would be expected to emphasize the emergence of functional receptor molecules from the synthetic pathway, rather than track translocation of receptors to the cell surface. To quantify total receptor density (i.e. cell surface plus intracellular sites), Filipeanu et al. elected not to utilize RX821002 binding (presumably because it matched the magnitude of their 'cell surface' receptors) but instead quantified total receptor density using GFP-tagged receptors, which would comprise both functional and non-functional receptors. The results of Filipeanu et al. are consistent with a component of intracellular receptors being retained as a chaperone-linked misfolded protein in the ER that can be corrected by prolonged exposure to low temperatures and enable a slow increase in binding sites for RX821002 [32]. However, rapid cold-induced mobilization of mature α_{2C}-ARs from the trans-Golgi to the plasma membrane, as previously reported [39,59,60], would not be expected to alter the density of functional RX821002 binding sites. Because they are mature receptors, they should bind RX821002 when present on trans-Golgi or plasma membranes, and would have been detected using RX821002 in crude cell membrane preparations or intact cells [30,33,46]. Filipeanu et al. also demonstrated that prolonged exposure to cold (18 h, 30 °C) increased the density of RX821002 binding sites in density-purified plasma membranes and caused marked relocation of GFP-tagged α_{2C}-ARs to the plasma membrane [32]. This analysis was performed only after prolonged exposure to cold (18 h) and therefore cannot discount a rapid translocation of mature α_{2C}-ARs followed by a slower emergence of previously-misfolded receptors from the ER. Concomitant with the rapid cold-induced translocation of mature α_{2C}-ARs to the cell surface, Jeyaraj et al. demonstrated a rapid emergence of a functional response to α_{2C}-AR stimulation (inhibition of cyclic AMP accumulation) [39]. In contrast, Filipeanu et al. were unable to observe a functional response until after 6 to 18 h of cooling [32]. This suggests that either the expression of α_{2C}-ARs was insufficient to elicit a functional response until misfolded receptors had been released from the ER, or there are clear differences between these studies in the manner in which HEK 293 cells process α_{2C}-ARs. Indeed, in the study by Filipeanu et al., the predominant α_{2C}-ARs appear to be the immature ~50 kD species, which would be consistent with retention in the ER. Interestingly, the molecular size of this receptor did not change on exposure to prolonged cooling [32], which might suggest a lack of receptor maturation. Clearly, the marked differences in behavior and characteristics of the α_{2C}-ARs in different studies of HEK 293 cells suggest that changes in experimental approach and handling of these cells may alter the activity of chaperones in the biosynthetic pathway and the subcellular retention and localization of these receptors.

Filipeanu et al. also proposed that the rapid cold-induced amplification of α_{2C}-AR vasoconstriction in cutaneous arteries is mediated by correction of an inherent defect in receptor folding and ER retention [32]. The authors observed that short-term exposure to cold (1 h) or to HSP90 inhibition (30 min) amplified contraction of rat tail arteries to the selective α_2-AR agonist UK 14,304 [32]. The time course of these rapid vasomotor changes is obviously not consistent with slow cold-induced export of misfolded proteins, including α_{2C}-ARs, from the ER [32,85]. The authors did not confirm that the effects of the HSP90 inhibitor were mediated by α_{2C}-ARs nor did they rule out the possibility of non-specific changes in vascular reactivity. Indeed, the effects of the inhibitor were most marked at high concentrations of UK 14,304 that are known to activate α_1-ARs [83,92]. Rapid cold-induced amplification of α_{2C}-AR activity in the cutaneous circulation is more consistent with translocation of mature receptors from the trans-Golgi rather than the slow emergence of correctly-folded receptors from the ER. However, the mechanism identified by Fillipeanu et al. whereby prolonged exposure to low temperatures counters ER-retention of α_{2C}-ARs and causes a post-translational increase in α_{2C}-AR binding sites might contribute to more chronic changes in thermosensitivity and cutaneous blood flow.

As occuring with other GPCRs [87–89], α_{2C}-ARs can attain cell surface expression through oligomerization with distinct receptors. Prinster et al. analyzed 25 distinct GPCRs, but found that only β_2-ARs could facilitate cell surface localization and functional rescue of α_{2C}-ARs in HEK 293 cells detected using a cell surface ELISA [34]. This was associated with an increase in density of binding sites for the α_2-AR ligand, rauwolscine (using crude membrane preparations), which the authors considered likely reflected the reduced retention of immature α_{2C}-ARs in the ER [34]. It is not known if interaction with β_2-ARs alters the putative HSP90-mediated retention of α_{2C}-ARs in the ER. α_{2C}-ARs can also dimerize with α_{2A}-ARs, but this does not alter the cellular localization of the receptor [4] (see also Section 3.3).

3.3. Signaling domains

Early research highlighted the signaling events involved in desensitization and internalization of GPCRs, including phosphorylation of agonist-occupied receptors by G-protein-coupled receptor kinases (GRKs), recruitment of β-arrestin and subsequent capture and endocytosis of GPCRs by clathrin-coated pits [67,93,94]. Internalization of receptors was thought to merely be a key component of desensitization, with the receptors being delivered to lysosomes for degradation or being rapidly recycled within the endosomal system (separated from ligands, dephosphorylated) and returned to the plasma membrane. More recent research indicates that internalization of GPCRs bestows novel signaling characteristics on these receptors. The possibility for endosomal signaling was elegantly demonstrated in studies of receptor tyrosine kinases [94]. For example, internalization of activated NGF receptors (TrkA) and transport of receptor-containing endosomes within neurons is required for TrkA to initiate effective nuclear signaling [94,95]. Regarding GPCRs, in addition to facilitating receptor internalization, β-arrestin functions as a scaffolding protein to recruit components of the MAPK signaling pathway and facilitate ERK activation [94]. Internalized endosomal GPCRs are thought to be capable of maintained signaling through ERK, Akt, and adenylyl cyclase and to initiate cellular responses distinct from cell surface receptors [94,96,97]. The concept that internalized receptors are recycled directly back to the cell surface has also been challenged by the observation that internalized GPCRs can be directed to and retained within the trans-Golgi compartment, colocalizing with TGN38 [98,99]. Therefore, a mobilizeable pool of cell-surface receptors in distal Golgi compartments (Section 3.1) could be generated by retention of newly-synthesized receptors but also from capture of receptors cycling from the cell surface.

Although α_{2C}-ARs are expressed predominantly as an intracellular receptor, when present on the cell surface they are resistant to phosphorylation by GRKs resulting in reduced interaction with β-arrestin and decreased desensitization and internalization compared to other α_2-ARs [4,36,100–102]. It is not clear whether mobilization of α_{2C}-AR from intracellular stores in response to agonist stimulation (i.e. homologous sensitization) might also contribute to reduced sensitivity of α_{2C}-ARs to desensitization. Although oligomerization between α_{2A}-ARs and α_{2C}-ARs does not change the cellular localization of these receptors, the interaction alters the signaling characteristics of α_{2A}-AR and its interaction with β-arrestin [4]. As expected, based on sensitivity of the individual receptors to GRKs, norepinephrine treatment caused recruitment of β-arrestin-GFP to plasma membranes of cells expressing α_{2A}-ARs but not in separate cells expressing α_{2C}-ARs. Intriguingly, when α_{2C}-ARs were co-expressed with

α_{2A}-ARs norepinephrine-induced phosphorylation of α_{2A}-ARs and recruitment of β-arrestin was reduced [4]. Furthermore, although co-expression of α_{2C}-ARs with α_{2A}-ARs did not alter norepinephrine-induced ERK activation, it reduced norepinephrine-induced activation of Akt when compared to the α_{2A}-AR alone. Therefore, despite a limited access of α_{2C}-ARs to the cell surface, the presence of these receptors can dramatically reduce α_{2A}-AR desensitization and alter α_{2A}-AR activity. It is not clear whether these events are inter-related and whether the α_{2A}-AR relies on internalization and endosomal signaling for this Akt signaling response. BRET analysis demonstrated that α_{2C}-ARs and α_{2A}-ARs can also form homodimers although heterodimerization appears to be the favored process [4]. There was no identification of the α_{2C}-AR molecular species interacting with α_{2A}-ARs [4] or whether oligomerization was associated with increased maturation of the α_{2C}-ARs species. Certainly, α_{2C}-ARs homodimers appeared to focus on ~50 and ~100 kD species, which would be consistent with lack of maturation of these receptors [4].

As with internalizing endosomal receptors, functional GPCRs that are retained within the secretory pathway appear fully capable of initiating signaling responses following activation by appropriate agonists. Indeed, heterotrimeric G-proteins are assembled and incorporated into membranes in the ER and can be found on membranes of the ER, Golgi compartments, and endosomes [94]. Furthermore, GPCRs can associate with heterotrimeric G-proteins and effector enzymes within the ER [103,104]. Perhaps the most remarkable demonstration of signaling by receptors retained within the biosynthetic pathway is with mutant vasopressin 2 receptors (V2Rs) that are responsible for Nephrogenic Diabetes Insipidus (NDI). Most of NDI mutations result in inherently functional receptors that are however misfolded and as a result retained within the ER. Therapeutic strategies have been directed at using chemical or pharmacological chaperones to rescue the mutant V2Rs [42]. However, a recent study demonstrated that functional but misfolded mutant V2Rs that are retained in the ER can be activated by non-peptide agonists (but not vasopressin) at their intracellular location and can initiate normal cellular responses, including elevations in cyclic AMP and modulation of Aquaporin-2 activity [43]. The receptor agonists did not alter the intracellular localization of the mutant receptors and did not promote maturation of the receptor species. Indeed, as a result of their intracellular localization, the intracellular receptors were not subject to the normal desensitization and degradation occurring with wild-type V2Rs [43]. It is not yet clear if this phenomenon can occur with intracellular α_{2C}-ARs, either by physiological or pharmacological means. The classical physiological agonists for α_{2C}-ARs, norepinephrine or epinephrine, are normally restricted to the extracellular environment. However, these agonists can gain access to the intracellular environment during catecholamine biosynthesis and during neuronal or extraneuronal uptake. Depending on the spatial relationship between intracellular α_{2C}-ARs, catecholamine uptake/biosynthetic pathways and catecholamine degradative enzymes, this might provide an additional mechanism for α_{2C}-AR activation and regulation of cellular responses. α_2-ARs may also be activated by non-traditional pathways. For example, α_2-ARs can be stimulated by L-arginine and its metabolites [105] providing an alternate route for activation of intracellular α_{2C}-ARs.

Intracellular GPCRs including α_{2C}-ARs provide cells with the ability to rapidly alter the nature or magnitude of cellular response to agonist stimulation (Fig. 1). These receptors should not be characterized solely in terms of storage depots, stunted development or signaling domains. Rather, they can function simultaneously within all three categories (Fig. 1).

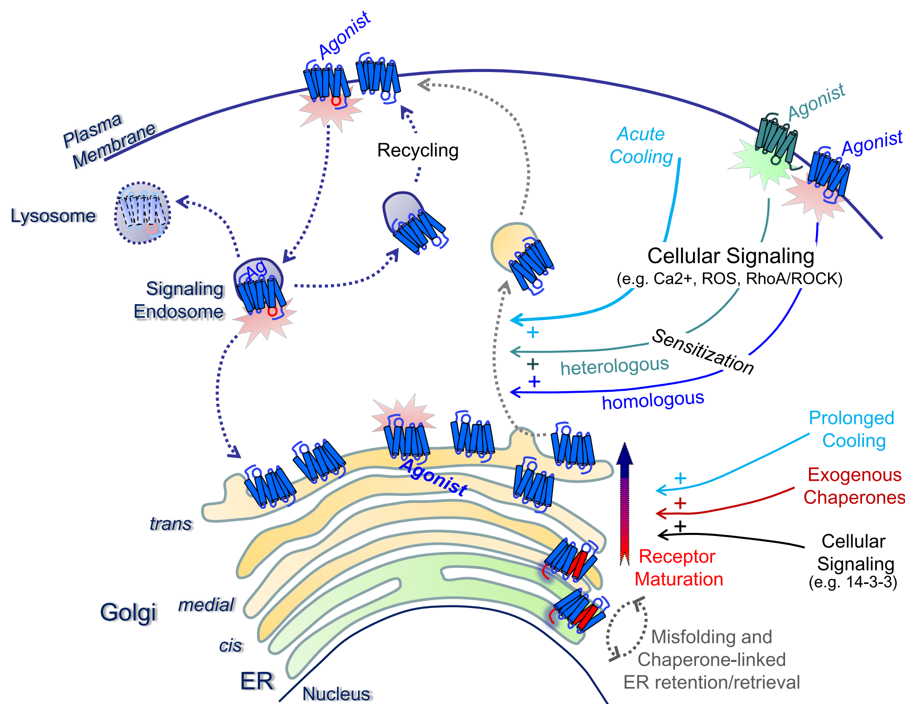

Fig. 1. Regulation of intracellular GPCR (including α_{2C}-ARs) activity. Newly synthesized GPCRs can be retained in the ER as a result of misfolding and/or chaperone-linked quality control and retention/retrieval mechanisms (designated as red 5th and 6th transmembrane domains). Prolonged cooling, cell signaling or exogenous chaperones (chemical, pharmacological) can counteract quality control and chaperone-mediated retention of GPCRs in the ER, enabling transport of the receptors through the Golgi compartments. Receptor maturation is associated with alterations in the glycosylation of the proteins, and acquisition of EndoH-resistance (designated as changes in GPCR N-terminus). Mature GPCRs can be retained in the trans-Golgi compartment for rapid transport to the cell surface in response to cell signaling activated by acute cooling, and by agonists stimulating the same GPCR (homologous) or distinct GPCRs (heterologous sensitization). Intracellular receptors retained in the biosynthetic pathway can be activated by agonists capable of accessing the intracellular compartments (GPCR activation designated by starbursts). Cell surface receptors can also be translocated to the trans-Golgi compartment following phosphorylation (by GRKs, designated by red 3rd intracellular loop) and internalization via the endosomal pathway.

Acknowledgements

This study was supported in part by grants from the NIH/National Heart, Lung, and Blood Institute (R21HL088087, MAC; R01HL080119, NAF) and the American Heart Association Great Rivers Affiliate (Beginning Grant-in-Aid, 0765204B, MAC).

References

[1] J.W. Regan, T.S. Kobilka, T.L. Yang-Feng, M.G. Caron, R.J. Lefkowitz, B.K. Kobilka, Cloning and expression of a human kidney cDNA for an alpha 2-adrenergic receptor subtype, Proc Natl Acad Sci U S A 85 (1988) 6301–6305.
[2] S. Schaak, J.C. Devedjian, C. Cayla, Y. Sender, H. Paris, Molecular cloning, sequencing and functional study of the promoter region of the human alpha2C4-adrenergic receptor gene, Biochem J 328 (Pt 2) (1997) 431–438.
[3] B.D. Bond RA, D.C. Eikenburg, J.P. Hieble, R. Hills, K.P. Minneman, S. Parra, "Adrenoceptors: α2C-adrenoceptor.", IUPHAR database (IUPHAR-DB), http://www.iuphar-db.org/DATABASE/ObjectDisplayForward?objectId=27. Last modified on 2010-06-28.
[4] K.M. Small, M.R. Schwarb, C. Glinka, C.T. Theiss, K.M. Brown, C.A. Seman, S.B. Liggett, Alpha2A- and alpha2C-adrenergic receptors form homo- and heterodimers: the heterodimeric state impairs agonist-promoted GRK phosphorylation and beta-arrestin recruitment, Biochemistry 45 (2006) 4760–4767.
[5] L. Hein, J.D. Altman, B.K. Kobilka, Two functionally distinct alpha2-adrenergic receptors regulate sympathetic neurotransmission, Nature 402 (1999) 181–184.
[6] M. Brede, F. Wiesmann, R. Jahns, K. Hadamek, C. Arnolt, S. Neubauer, M.J. Lohse, L. Hein, Feedback inhibition of catecholamine release by two different alpha2-adrenoceptor subtypes prevents progression of heart failure, Circulation 106 (2002) 2491–2496.
[7] K.M. Small, S.L. Forbes, F.F. Rahman, K.M. Bridges, S.B. Liggett, A four amino acid deletion polymorphism in the third intracellular loop of the human alpha 2 C-adrenergic receptor confers impaired coupling to multiple effectors, J Biol Chem 275 (2000) 23059–23064.
[8] K.M. Small, L.E. Wagoner, A.M. Levin, S.L. Kardia, S.B. Liggett, Synergistic polymorphisms of beta1- and alpha2C-adrenergic receptors and the risk of congestive heart failure, N Engl J Med 347 (2002) 1135–1142.
[9] K.M. Small, J. Mialet-Perez, C.A. Seman, C.T. Theiss, K.M. Brown, S.B. Liggett, Polymorphisms of cardiac presynaptic alpha2C adrenergic receptors: diverse intragenic variability with haplotype-specific functional effects, Proc Natl Acad Sci U S A 101 (2004) 13020–13025.
[10] M. Scheinin, J. Sallinen, A. Haapalinna, Evaluation of the alpha2C-adrenoceptor as a neuropsychiatric drug target studies in transgenic mouse models, Life Sci 68 (2001) 2277–2285.
[11] P.P. Lakhlani, L.B. MacMillan, T.Z. Guo, B.A. McCool, D.M. Lovinger, M. Maze, L.E. Limbird, Substitution of a mutant alpha2a-adrenergic receptor via "hit and run" gene targeting reveals the role of this subtype in sedative, analgesic, and anesthetic-sparing responses in vivo, Proc Natl Acad Sci U S A 94 (1997) 9950–9955.
[12] J.D. Altman, A.U. Trendelenburg, L. MacMillan, D. Bernstein, L. Limbird, K. Starke, B.K. Kobilka, L. Hein, Abnormal regulation of the sympathetic nervous system in alpha2A-adrenergic receptor knockout mice, Mol Pharmacol 56 (1999) 154–161.
[13] S. Guimaraes, D. Moura, Vascular adrenoceptors: an update, Pharmacol Rev 53 (2001) 319–356.
[14] N.A. Flavahan, L.E. Lindblad, T.J. Verbeuren, J.T. Shepherd, P.M. Vanhoutte, Cooling and alpha 1- and alpha 2-adrenergic responses in cutaneous veins: role of receptor reserve, Am J Physiol 249 (1985) H950–H955.
[15] J. Shepard, P. Vanhoutte, The human cardiovascular system: facts and concepts, Raven Press, New York, 1979.
[16] M.A. Chotani, S. Mitra, B.Y. Su, S. Flavahan, A.H. Eid, K.R. Clark, C.R. Montague, H. Paris, D.E. Handy, N.A. Flavahan, Regulation of alpha(2)-adrenoceptors in human vascular smooth muscle cells, Am J Physiol Heart Circ Physiol 286 (2004) H59–H67.
[17] E. MacDonald, B.K. Kobilka, M. Scheinin, Gene targeting-homing in on alpha 2-adrenoceptor-subtype function, Trends Pharmacol Sci 18 (1997) 211–219.
[18] R.E. Link, K. Desai, L. Hein, M.E. Stevens, A. Chruscinski, D. Bernstein, G.S. Barsh, B.K. Kobilka, Cardiovascular regulation in mice lacking alpha2-adrenergic receptor subtypes b and c, Science 273 (1996) 803–805.
[19] L.B. MacMillan, L. Hein, M.S. Smith, M.T. Piascik, L.E. Limbird, Central hypotensive effects of the alpha2a-adrenergic receptor subtype, Science 273 (1996) 801–803.
[20] M.A. Chotani, S. Flavahan, S. Mitra, D. Daunt, N.A. Flavahan, Silent alpha(2 C)-adrenergic receptors enable cold-induced vasoconstriction in cutaneous arteries, Am J Physiol Heart Circ Physiol 278 (2000) H1075–H1083.
[21] P. Vanhoutte, Physical factors of regulation, in: S.A. Bohr DF, H.V. Sparks Jr. (Eds.), Handbook of Physiology, Section 2: The Cardiovascular System, vol. II, Vascular Smooth Muscle American Physiological Society, Bethesda, MD, 1980, pp. 443–474.
[22] M. Honda, M. Suzuki, K. Nakayama, T. Ishikawa, Role of alpha2C-adrenoceptors in the reduction of skin blood flow induced by local cooling in mice, Br J Pharmacol 152 (2007) 91–100.
[23] C.S. Thompson-Torgerson, L.A. Holowatz, N.A. Flavahan, W.L. Kenney, Cold-induced cutaneous vasoconstriction is mediated by Rho kinase in vivo in human skin, Am J Physiol Heart Circ Physiol 292 (2007) H1700–H1705.
[24] M.A. Chotani, S. Mitra, A.H. Eid, S.A. Han, N.A. Flavahan, Distinct cAMP signaling pathways differentially regulate alpha2C-adrenoceptor expression: role in serum induction in human arteriolar smooth muscle cells, Am J Physiol Heart Circ Physiol 288 (2005) H69–H76.
[25] A.H. Eid, M.A. Chotani, S. Mitra, T.J. Miller, N.A. Flavahan, Cyclic AMP acts through Rap1 and JNK signaling to increase expression of cutaneous smooth muscle alpha2C-adrenoceptors, Am J Physiol Heart Circ Physiol 295 (2008) H266–H272.
[26] N.A. Flavahan, S. Flavahan, S. Mitra, M.A. Chotani, The vasculopathy of Raynaud's phenomenon and scleroderma, Rheum Dis Clin North Am 29 (2003) 275–291 vi.
[27] D.L. Rosin, E.M. Talley, A. Lee, R.L. Stornetta, B.D. Gaylinn, P.G. Guyenet, K.R. Lynch, Distribution of alpha 2 C-adrenergic receptor-like immunoreactivity in the rat central nervous system, J Comp Neurol 372 (1996) 135–165.
[28] P.C. Brum, C.M. Hurt, O.G. Shcherbakova, B. Kobilka, T. Angelotti, Differential targeting and function of alpha2A and alpha2C adrenergic receptor subtypes in cultured sympathetic neurons, Neuropharmacology 51 (2006) 397–413.
[29] J.G. Richman, J.W. Regan, Alpha 2-adrenergic receptors increase cell migration and decrease F-actin labeling in rat aortic smooth muscle cells, Am J Physiol 274 (1998) C654–C662.
[30] T. Angelotti, D. Daunt, O.G. Shcherbakova, B. Kobilka, C.M. Hurt, Regulation of G-protein coupled receptor traffic by an evolutionary conserved hydrophobic signal, Traffic 11 (2010) 560–578.
[31] D.A. Daunt, C. Hurt, L. Hein, J. Kallio, F. Feng, B.K. Kobilka, Subtype-specific intracellular trafficking of alpha2-adrenergic receptors, Mol Pharmacol 51 (1997) 711–720.
[32] C.M. Filipeanu, R. de Vries, A.H. Danser, D.R. Kapusta, Modulation of alpha(2 C) adrenergic receptor temperature-sensitive trafficking by HSP90, Biochim Biophys Acta 1813 (2011) 346–357.
[33] C.M. Hurt, F.Y. Feng, B. Kobilka, Cell-type specific targeting of the alpha 2c-adrenoceptor. Evidence for the organization of receptor microdomains during neuronal differentiation of PC12 cells, J Biol Chem 275 (2000) 35424–35431.
[34] S.C. Prinster, T.G. Holmqvist, R.A. Hall, Alpha2C-adrenergic receptors exhibit enhanced surface expression and signaling upon association with beta2-adrenergic receptors, J Pharmacol Exp Ther 318 (2006) 974–981.
[35] M. Wozniak, L.E. Limbird, The three alpha 2-adrenergic receptor subtypes achieve basolateral localization in Madin-Darby canine kidney II cells via different targeting mechanisms, J Biol Chem 271 (1996) 5017–5024.
[36] H. Kurose, R.J. Lefkowitz, Differential desensitization and phosphorylation of three cloned and transfected alpha 2-adrenergic receptor subtypes, J Biol Chem 269 (1994) 10093–10099.
[37] A. Velasco, L. Hendricks, K.W. Moremen, D.R. Tulsiani, O. Touster, M.G. Farquhar, Cell type-dependent variations in the subcellular distribution of alpha-mannosidase I and II, J Cell Biol 122 (1993) 39–51.
[38] A. Herscovics, Importance of glycosidases in mammalian glycoprotein biosynthesis, Biochim Biophys Acta 1473 (1999) 96–107.
[39] S.C. Jeyaraj, M.A. Chotani, S. Mitra, H.E. Gregg, N.A. Flavahan, K.J. Morrison, Cooling evokes redistribution of alpha2C-adrenoceptors from Golgi to plasma membrane in transfected human embryonic kidney 293 cells, Mol Pharmacol 60 (2001) 1195–1200.
[40] C.R. Brown, L.Q. Hong-Brown, J. Biwersi, A.S. Verkman, W.J. Welch, Chemical chaperones correct the mutant phenotype of the delta F508 cystic fibrosis transmembrane conductance regulator protein, Cell Stress Chaperones 1 (1996) 117–125.
[41] X. Wang, A.V. Koulov, W.A. Kellner, J.R. Riordan, W.E. Balch, Chemical and biological folding contribute to temperature-sensitive DeltaF508 CFTR trafficking, Traffic 9 (2008) 1878–1893.
[42] J.H. Robben, P.M. Deen, Pharmacological chaperones in nephrogenic diabetes insipidus: possibilities for clinical application, BioDrugs 21 (2007) 157–166.
[43] J.H. Robben, M.L. Kortenoeven, M. Sze, C. Yae, G. Milligan, V.M. Oorschot, J. Klumperman, N.V. Knoers, P.M. Deen, Intracellular activation of vasopressin V2 receptor mutants in nephrogenic diabetes insipidus by nonpeptide agonists, Proc Natl Acad Sci U S A 106 (2009) 12195–12200.
[44] F. Zhou, C.M. Filipeanu, M.T. Duvernay, G. Wu, Cell-surface targeting of alpha2-adrenergic receptors – inhibition by a transport deficient mutant through dimerization, Cell Signal 18 (2006) 318–327.
[45] C.M. Filipeanu, Temperature-dependent interactions of alpha2C-adrenergic receptor with molecular chaperones, Circulation 118 (2008) s514.
[46] A. Lee, E. Talley, D.L. Rosin, K.R. Lynch, Characterization of alpha 2A-adrenergic receptors in GT1 neurosecretory cells, Neuroendocrinology 62 (1995) 215–225.
[47] D. Ma, L.Y. Jan, ER transport signals and trafficking of potassium channels and receptors, Curr Opin Neurobiol 12 (2002) 287–292.
[48] B. Schwappach, N. Zerangue, Y.N. Jan, L.Y. Jan, Molecular basis for K(ATP) assembly: transmembrane interactions mediate association of a K + channel with an ABC transporter, Neuron 26 (2000) 155–167.
[49] P. Vivithanaporn, S. Yan, G.T. Swanson, Intracellular trafficking of KA2 kainate receptors mediated by interactions with coatomer protein complex I (COPI) and 14–3–3 chaperone systems, J Biol Chem 281 (2006) 15475–15484.
[50] I. O'Kelly, M.H. Butler, N. Zilberberg, S.A. Goldstein, Forward transport. 14–3–3 binding overcomes retention in endoplasmic reticulum by dibasic signals, Cell 111 (2002) 577–588.
[51] T. Olli-Lahdesmaki, J. Kallio, M. Scheinin, Receptor subtype-induced targeting and subtype-specific internalization of human alpha(2)-adrenoceptors in PC12 cells, J Neurosci 19 (1999) 9281–9288.
[52] R.D. Teasdale, F. Matheson, P.A. Gleeson, Post-translational modifications distinguish cell surface from Golgi-retained beta 1,4 galactosyltransferase molecules, Golgi localization involves active retention, Glycobiology 4 (1994) 917–928.
[53] J. Wu, N.J. Chalupny, T.J. Manley, S.R. Riddell, D. Cosman, T. Spies, Intracellular retention of the MHC class I-related chain B ligand of NKG2D by the human cytomegalovirus UL16 glycoprotein, J Immunol 170 (2003) 4196–4200.

[54] F.J. Eng, O. Varlamov, L.D. Fricker, Sequences within the cytoplasmic domain of gp180/carboxypeptidase D mediate localization to the trans-Golgi network, Mol Biol Cell 10 (1999) 35–46.
[55] J.M. Wahlberg, I. Geffen, F. Reymond, T. Simmen, M. Spiess, Trans-Golgi retention of a plasma membrane protein: mutations in the cytoplasmic domain of the asialoglycoprotein receptor subunit H1 result in trans-Golgi retention, J Cell Biol 130 (1995) 285–297.
[56] D. Aoki, N. Lee, N. Yamaguchi, C. Dubois, M.N. Fukuda, Golgi retention of a trans-Golgi membrane protein, galactosyltransferase, requires cysteine and histidine residues within the membrane-anchoring domain, Proc Natl Acad Sci U S A 89 (1992) 4319–4323.
[57] Z.H. Wang, M.D. Gershon, O. Lungu, Z. Zhu, A.A. Gershon, Trafficking of varicella-zoster virus glycoprotein gI: T(338)-dependent retention in the trans-Golgi network, secretion, and mannose 6-phosphate-inhibitable uptake of the ectodomain, J Virol 74 (2000) 6600–6613.
[58] L. Braiterman, L. Nyasae, Y. Guo, R. Bustos, S. Lutsenko, A. Hubbard, Apical targeting and Golgi retention signals reside within a 9-amino acid sequence in the copper-ATPase, ATP7B, Am J Physiol Gastrointest Liver Physiol 296 (2009) G433–G444.
[59] S.R. Bailey, S. Mitra, S. Flavahan, N.A. Flavahan, Reactive oxygen species from smooth muscle mitochondria initiate cold-induced constriction of cutaneous arteries, Am J Physiol Heart Circ Physiol 289 (2005) H243–H250.
[60] S.R. Bailey, A.H. Eid, S. Mitra, S. Flavahan, N.A. Flavahan, Rho kinase mediates cold-induced constriction of cutaneous arteries: role of alpha2C-adrenoceptor translocation, Circ Res 94 (2004) 1367–1374.
[61] L. Hein, K. Ishii, S.R. Coughlin, B.K. Kobilka, Intracellular targeting and trafficking of thrombin receptors. A novel mechanism for resensitization of a G protein-coupled receptor, J Biol Chem 269 (1994) 27719–27726.
[62] O. Seksek, J. Biwersi, A.S. Verkman, Direct measurement of trans-Golgi pH in living cells and regulation by second messengers, J Biol Chem 270 (1995) 4967–4970.
[63] E.H. Pap, T.B. Dansen, R. van Summeren, K.W. Wirtz, Peptide-based targeting of fluorophores to organelles in living cells, Exp Cell Res 265 (2001) 288–293.
[64] A.G. Roseberry, M. Bunemann, J. Elavunkal, M.M. Hosey, Agonist-dependent delivery of M(2) muscarinic acetylcholine receptors to the cell surface after pertussis toxin treatment, Mol Pharmacol 59 (2001) 1256–1268.
[65] S. Mittar, C. Ulyatt, G.J. Howell, A.F. Bruns, I. Zachary, J.H. Walker, S. Ponnambalam, VEGFR1 receptor tyrosine kinase localization to the Golgi apparatus is calcium-dependent, Exp Cell Res 315 (2009) 877–889.
[66] K.A. Kim, M. von Zastrow, Neurotrophin-regulated sorting of opioid receptors in the biosynthetic pathway of neurosecretory cells, J Neurosci 23 (2003) 2075–2085.
[67] N.J. Freedman, R.J. Lefkowitz, Desensitization of G protein-coupled receptors, Recent Prog Horm Res 51 (1996) 319–351; discussion 352–313.
[68] N.A. Flavahan, The role of alpha2-adrenoceptors as cutaneous thermosensors, News in Physiological Sciences 6 (1991) 251–255.
[69] S. Guimaraes, D. Moura, Vascular adrenoceptors: an update, Pharmacol Rev 53 (2001) 319–356.
[70] F.M. Wigley, N.A. Flavahan, Raynaud's phenomenon, Rheum Dis Clin North Am 22 (1996) 765–781.
[71] L. Ekenvall, L.E. Lindblad, O. Norbeck, B.M. Etzell, Alpha-Adrenoceptors and cold-induced vasoconstriction in human finger skin, Am J Physiol 255 (1988) H1000–H1003.
[72] J.E. Faber, Effect of local tissue cooling on microvascular smooth muscle and postjunctional alpha 2-adrenoceptors, Am J Physiol 255 (1988) H121–H130.
[73] C.T. Harker, P.J. Ousley, C.J. Bowman, J.M. Porter, Cooling augments alpha 2-adrenoceptor-mediated contractions in rat tail artery, Am J Physiol 260 (1991) H1166–H1171.
[74] C.T. Harker, P.J. Ousley, E.J. Harris, J.M. Edwards, L.M. Taylor, J.M. Porter, The effects of cooling on human saphenous vein reactivity to adrenergic agonists, J Vasc Surg 12 (1990) 45–49.
[75] R.R. Freedman, R.P. Baer, M.D. Mayes, Blockade of vasospastic attacks by alpha 2-adrenergic but not alpha 1- adrenergic antagonists in idiopathic Raynaud's disease, Circulation 92 (1995) 1448–1451.
[76] L.E. Lindblad, L. Ekenvall, Alpha 2-adrenoceptor inhibition in patients with vibration white fingers, Kurume Med J 37 (Suppl) (1990) S95–S99.
[77] N.A. Flavahan, Phenylpropanolamine constricts mouse and human blood vessels by preferentially activating alpha2-adrenoceptors, J Pharmacol Exp Ther 313 (2005) 432–439.
[78] N.A. Flavahan, T.J. Rimele, J.P. Cooke, P.M. Vanhoutte, Characterization of postjunctional alpha-1 and alpha-2 adrenoceptors activated by exogenous or nerve-released norepinephrine in the canine saphenous vein, J Pharmacol Exp Ther 230 (1984) 699–705.
[79] N.A. Flavahan, P.M. Vanhoutte, Alpha-1 and alpha-2 adrenoceptor: response coupling in canine saphenous and femoral veins, J Pharmacol Exp Ther 238 (1986) 131–138.
[80] W.R. Dunn, J.C. McGrath, V.G. Wilson, Influence of angiotensin II on the alpha-adrenoceptors involved in mediating the response to sympathetic nerve stimulation in the rabbit isolated distal saphenous artery, Br J Pharmacol 102 (1991) 10–12.
[81] A.G. Templeton, J. Macmillan, J.C. McGrath, N.D. Storey, V.G. Wilson, Evidence for prazosin-resistant, rauwolscine-sensitive alpha-adrenoceptors mediating contractions in the isolated vascular bed of the rat tail, Br J Pharmacol 97 (1989) 563–571.
[82] X.H. Xiao, M.J. Rand, Vasoconstrictor responses to the alpha 2-adrenoceptor agonist UK14304 during responses to noradrenaline, phenylephrine, serotonin and vasopressin of the rat tail artery, Arch Int Pharmacodyn Ther 308 (1990) 63–75.
[83] F. Jantschak, A.M. Popp, R.A. Hofmann, C.M. Villalon, D. Centurion, H.H. Pertz, Postjunctional alpha2C-adrenoceptors mediate vasoconstriction in rat tail artery: influence of precontraction and temperature on vasoreactivity, Naunyn Schmiedebergs Arch Pharmacol 382 (2010) 487–497.
[84] J.M. Hughes, O. Wirth, K. Krajnak, R. Miller, S. Flavahan, D.E. Berkowitz, D. Welcome, N.A. Flavahan, Increased oxidant activity mediates vascular dysfunction in vibration injury, J Pharmacol Exp Ther 328 (2009) 223–230.
[85] G.M. Denning, M.P. Anderson, J.F. Amara, J. Marshall, A.E. Smith, M.J. Welsh, Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive, Nature 358 (1992) 761–764.
[86] J.L. Delaunay, A.M. Durand-Schneider, D. Delautier, A. Rada, J. Gautherot, E. Jacquemin, T. Ait-Slimane, M. Maurice, A missense mutation in ABCB4 gene involved in progressive familial intrahepatic cholestasis type 3 leads to a folding defect that can be rescued by low temperature, Hepatology 49 (2009) 1218–1227.
[87] C. Hague, S.E. Lee, Z. Chen, S.C. Prinster, R.A. Hall, K.P. Minneman, Heterodimers of alpha1B- and alpha1D-adrenergic receptors form a single functional entity, Mol Pharmacol 69 (2006) 45–55.
[88] S.C. Prinster, C. Hague, R.A. Hall, Heterodimerization of g protein-coupled receptors: specificity and functional significance, Pharmacol Rev 57 (2005) 289–298.
[89] M.A. Uberti, C. Hague, H. Oller, K.P. Minneman, R.A. Hall, Heterodimerization with beta2-adrenergic receptors promotes surface expression and functional activity of alpha1D-adrenergic receptors, J Pharmacol Exp Ther 313 (2005) 16–23.
[90] X. Wang, J. Venable, P. LaPointe, D.M. Hutt, A.V. Koulov, J. Coppinger, C. Gurkan, W. Kellner, J. Matteson, H. Plutner, J.R. Riordan, J.W. Kelly, J.R. Yates 3rd, W.E. Balch, Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis, Cell 127 (2006) 803–815.
[91] F. Sun, Z. Mi, S.B. Condliffe, C.A. Bertrand, X. Gong, X. Lu, R. Zhang, J.D. Latoche, J.M. Pilewski, P.D. Robbins, R.A. Frizzell, Chaperone displacement from mutant cystic fibrosis transmembrane conductance regulator restores its function in human airway epithelia, FASEB J 22 (2008) 3255–3263.
[92] N.L. Kanagy, Increased vascular responsiveness to alpha 2-adrenergic stimulation during NOS inhibition-induced hypertension, Am J Physiol 273 (1997) H2756–H2764.
[93] C.M. Tan, A.E. Brady, H.H. Nickols, Q. Wang, L.E. Limbird, Membrane trafficking of G protein-coupled receptors, Annu Rev Pharmacol Toxicol 44 (2004) 559–609.
[94] D. Calebiro, V.O. Nikolaev, L. Persani, M.J. Lohse, Signaling by internalized G-protein-coupled receptors, Trends Pharmacol Sci 31 (2010) 221–228.
[95] L.S. Zweifel, R. Kuruvilla, D.D. Ginty, Functions and mechanisms of retrograde neurotrophin signalling, Nat Rev Neurosci 6 (2005) 615–625.
[96] F. Gobeil, A. Fortier, T. Zhu, M. Bossolasco, M. Leduc, M. Grandbois, N. Heveker, G. Bkaily, S. Chemtob, D. Barbaz, G-protein-coupled receptors signalling at the cell nucleus: an emerging paradigm, Can J Physiol Pharmacol 84 (2006) 287–297.
[97] A. Valdehita, A.M. Bajo, A.B. Fernandez-Martinez, M.I. Arenas, E. Vacas, P. Valenzuela, A. Ruiz-Villaespesa, J.C. Prieto, M.J. Carmena, Nuclear localization of vasoactive intestinal peptide (VIP) receptors in human breast cancer, Peptides 31 (2010) 2035–2045.
[98] J.M. Escola, G. Kuenzi, H. Gaertner, M. Foti, O. Hartley, CC chemokine receptor 5 (CCR5) desensitization: cycling receptors accumulate in the trans-Golgi network, J Biol Chem 285 (2010) 41772–41780.
[99] B. Lelouvier, G. Tamagno, A.M. Kaindl, A. Roland, V. Lelievre, V. Le Verche, C. Loudes, P. Gressens, A. Faivre-Baumann, Z. Lenkei, P. Dournaud, Dynamics of somatostatin type 2A receptor cargoes in living hippocampal neurons, J Neurosci 28 (2008) 4336–4349.
[100] E.A. Jewell-Motz, S.B. Liggett, G protein-coupled receptor kinase specificity for phosphorylation and desensitization of alpha2-adrenergic receptor subtypes, J Biol Chem 271 (1996) 18082–18087.
[101] M.G. Eason, S.B. Liggett, Functional alpha 2-adrenergic receptor-Gs coupling undergoes agonist-promoted desensitization in a subtype-selective manner, Biochem Biophys Res Commun 193 (1993) 318–323.
[102] M.G. Eason, S.B. Liggett, Subtype-selective desensitization of alpha 2-adrenergic receptors. Different mechanisms control short and long term agonist-promoted desensitization of alpha 2 C10, alpha 2 C4, and alpha 2C2, J Biol Chem 267 (1992) 25473–25479.
[103] D.J. Dupre, A. Baragli, R.V. Rebois, N. Ethier, T.E. Hebert, Signalling complexes associated with adenylyl cyclase II are assembled during their biosynthesis, Cell Signal 19 (2007) 481–489.
[104] D.J. Dupre, M. Robitaille, N. Ethier, L.R. Villeneuve, A.M. Mamarbachi, T.E. Hebert, Seven transmembrane receptor core signaling complexes are assembled prior to plasma membrane trafficking, J Biol Chem 281 (2006) 34561–34573.
[105] M.S. Joshi, T.B. Ferguson Jr., F.K. Johnson, R.A. Johnson, S. Parthasarathy, J.R. Lancaster Jr., Receptor-mediated activation of nitric oxide synthesis by arginine in endothelial cells, Proc Natl Acad Sci U S A 104 (2007) 9982–9987.