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β-Adrenergic receptors and their interacting proteins

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Abstract

The three subtypes of β-adrenergic receptor (βAR) all interact with G proteins as a central aspect of their signaling. The various βAR subtypes also associate differentially with a variety of other cytoplasmic and transmembrane proteins. These βAR-interacting proteins play distinct roles in the regulation of receptor signaling and trafficking. The specificity of βAR associations with various binding partners can help to explain key physiological differences between βAR subtypes. Moreover, the differential tissue expression patterns of many of the βAR-interacting proteins may contribute to tissue-specific regulation of βAR function.

Keywords: Beta; Adrenoceptor; PDZ; GPCR; Heptahelical

1. Introduction

β-Adrenergic receptors (βARs) are G protein-coupled receptors (GPCRs) that mediate physiological responses to adrenaline and noradrenaline. These receptors are the molecular targets for some of the most commonly prescribed drugs in the history of medicine. βAR antagonists ("beta blockers") are routinely used in the treatment of heart disease and hypertension, and β_2AR agonists, such as albuterol, are commonly used in the treatment of asthma. There are three receptor subtypes in this family: β_1AR is found at its highest levels in the heart and brain [1], β_2AR is more widely expressed [2], and β_3AR is found at its highest levels in adipose tissue [3]. All three receptors couple primarily to $G\alpha_s$ to stimulate adenylyl cyclase, but can also couple to $G\alpha_i$ in some cells under certain conditions [4–6].

The first proteins found to have functional interactions with βARs were, of course, G proteins [7]. Next was a kinase, originally called the β-adrenergic receptor kinase (βARK) [8] and now known as G protein-coupled receptor kinase 2 (GRK2). This kinase belongs to a family with seven closely related members, most of which are capable of associating with and phosphorylating β_1AR and β_2AR [9] but not β_3AR [10]. In a similar vein, β_1AR [11] and β_2AR [12], but not β_3AR [13], can be phosphorylated by protein kinase A (PKA), which can lead to feedback desensitization of βAR signaling, since activation of PKA is a downstream consequence of βAR-mediated adenylyl cyclase stimulation [14]. Finally, β-arrestins were identified as proteins involved in the desensitization of β_2AR [15,16] and are also known to functionally interact with β_1AR [11] but not β_3AR [13] to regulate receptor internalization and desensitization in a manner that is dependent on GRK-mediated phosphorylation [17].

β_1AR and β_2AR share 54% sequence identity and are expressed in many of the same tissues. Furthermore, by the mid-1990s, the sets of cytoplasmic partners with which these two receptors were known to associate were exactly the same: $G\alpha_s$, GRKs, PKA, and β-arrestins (as described above). Paradoxically, however, physiological studies of the same era revealed that β_1AR and β_2AR could exert markedly different functional effects even when expressed in the same cell types. In cardiac myocytes, for example, β_1AR and β_2AR are expressed at similar levels and induce similar rises in cyclic AMP, yet exert quite different effects on the regulation of cellular calcium levels and contractility [18–20]. Furthermore, β_1AR and β_2AR also have opposing effects on the regulation of apoptosis in a variety of cell types [21–24] and can differentially regulate calcium channel activity in adrenal chromaffin cells [25]. Studies with knockout mice have revealed significantly different phenotypes for β_1AR versus β_2AR knockouts [26,27], further suggesting that the two receptors are capable of coupling to distinct intracellular signaling pathways.

The many differences observed between the effects of β_1AR and β_2AR on cellular physiology are inconsistent with the notion that the two receptors couple to precisely the same set of intracellular signaling proteins. Thus, over the past several years, a number of research groups have pur-

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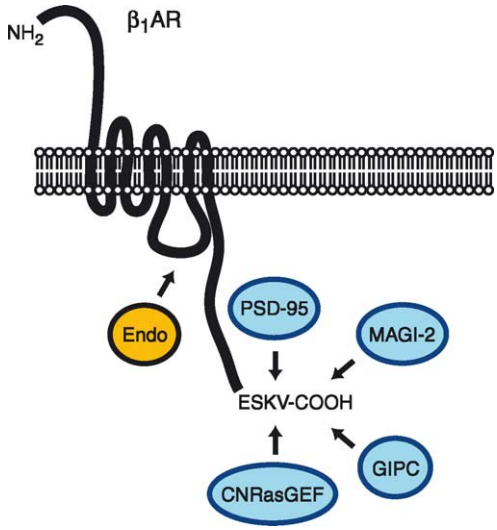

Fig. 1. Cytoplasmic proteins that associate selectively with β_1AR. Endophilins (shown in orange) interact with a proline-rich motif on the β_1AR third intracellular loop, while four different PDZ proteins (shown in blue) interact with the E-S-K-V motif found at the β_1AR carboxyl-terminus.

sued studies aimed at elucidating novel βAR-binding partners. A special emphasis has been placed on finding proteins that interact differentially with β_1AR versus β_2AR and may therefore potentially contribute toward understanding the differential signaling and regulation of these two subtypes. This review will describe what is currently known about the subtype-specific interactions of βARs with various cytoplasmic proteins and will also summarize findings concerning βAR associations with transmembrane proteins, such as receptors and channels (Figs. 1–4).

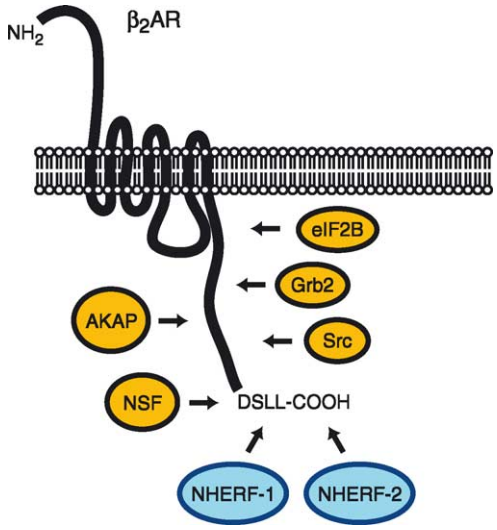

Fig. 2. Cytoplasmic proteins that associate selectively with β_2AR. The PDZ proteins NHERF-1 and NHERF-2 (shown in blue) interact with the D-S-L-L motif found at the β_2AR carboxyl-terminus. This motif is also recognized by NSF, which is not a PDZ protein (shown in orange). Other proteins that lack PDZ domains and but are known to associate with various regions of the β_2AR carboxyl-terminus include AKAPs, eIF-2B, Grb2, and Src.

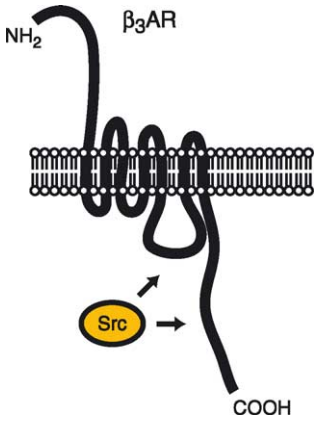

Fig. 3. Cytoplasmic proteins that associate selectively with β_3AR. Other than G proteins, the only cytoplasmic protein that is currently known to associate with β_3AR is Src, which interacts with proline-rich regions on the β_3AR third intracellular loop and carboxyl-terminus.

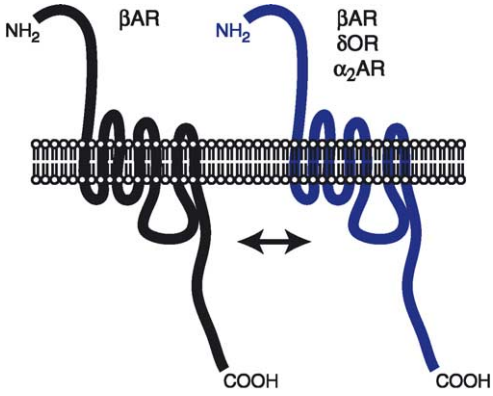

Fig. 4. Heterodimerization between β-adrenergic receptors and other GPCRs. β_1AR and β_2AR are both capable of homodimerization as well as heterodimerization with each other. βARs can also heterodimerize with other GPCRs, such as δ-opioid receptors and α_2-adrenergic receptors (shown in blue).

2. β_1-Adrenergic receptor-interacting proteins

The first cytoplasmic protein that was found to interact with β_1AR but not β_2AR was a Src homology (SH3) domain-containing protein, originally named SH3p4 and now called endophilin-1 [28]. SH3 domains are known to associate avidly with specific proline-rich motifs [29]. Indeed, endophilin-1 was found to associate with a proline-rich region of the cytoplasmic third loop of β_1AR [28]. This proline-rich region is not found in β_2AR, which explains the selective interaction of endophilin-1 with β_1AR but not β_2AR. Coincidentally, the interaction of endophilin-1 with the β_1AR third cytoplasmic loop was pulled out simultaneously by two completely independent methods: the yeast two-hybrid system [30] and biochemical purification using a piece of the receptor expressed as a glutathione-*S*-transferase (GST) fusion protein [31]. Most GPCR interactions discovered to date have been pulled out using one or the other of these techniques, but it is rare that

an unbiased genetic screen and an unbiased biochemical purification yield exactly the same interacting partner.

When the β_1AR/endophilin-1 interaction was first reported, little was known about the function of endophilin-1 other than that it could bind to dynamin and synaptojanin [32,33]. The primary functional effect of endophilin-1 overexpression on β_1AR function was found to be a twofold enhancement in the rate of agonist-induced receptor internalization [28]. Interestingly, the proline-rich region of the β_1AR third loop where endophilin-1 binds had previously been shown to play a role in regulating receptor internalization [34]. The mechanism(s) by which endophilins might regulate β_1AR endocytosis are unknown, but they could potentially include (i) a direct allosteric effect on receptor conformation, (ii) an alteration of receptor interactions with β-arrestins, and/or (iii) linkage of the receptor to other proteins involved in the regulation of cellular trafficking, such as dynamin or synaptojanin. Over the past 3 years, much has been learned about the cellular functions of endophilin-1 [35], and this information may yet lead to further insights into the physiological importance of the β_1AR/endophilin-1 interaction.

In addition to endophilin-1, there have been a number of other proteins found to interact selectively with β_1AR but not β_2AR. All of these other proteins are known to interact with the β_1AR carboxyl-terminus (β_1AR-CT) and to possess PSD-95/Discs-large/ZO-1-homology (PDZ) domains, which are specialized protein–protein interaction domains capable of mediating associations with the carboxyl-termini of target proteins [36]. The first PDZ domain-containing protein that was found to interact with β_1AR was the post-synaptic density protein of 95 kDa (PSD-95) [37]. This protein is found most abundantly in the brain and has three PDZ domains [38]. The β_1AR/PSD-95 interaction was originally pulled out in a yeast two-hybrid screen, and is mediated via binding of the third PDZ domain of PSD-95 to the last few amino acids of the β_1AR (E-S-K-V) [37]. The terminal Val residue and the Ser at the −2 position are especially critical for the interaction. This distal portion of the carboxyl-terminus is not conserved amongst β-adrenergic receptors, thereby explaining the selective of interaction of PSD-95 with β_1AR. Association of PSD-95 with β_1AR was found to impair β_1AR internalization and to also facilitate the physical linkage of β_1AR to NMDA-type glutamate receptors [37], which are known to be regulated by β-adrenergic stimulation in the brain [39–41]. Moreover, PSD-95 is known to be concentrated at synapses [38] and may therefore play a role in the localization of β_1AR to synaptic regions in the brain [42,43].

Since the report of the β_1AR/PSD-95 association, several other PDZ protein interactions with β_1AR have been described. The first PDZ domain of the multi-PDZ protein known as membrane-associated guanylate kinase-like inverted-2 (MAGI-2), for example, was found to be a high-affinity binding partner for the β_1AR-CT [44]. The interaction of β_1AR with MAGI-2, which is also known as AIP1 [45] and S-SCAM [46], was originally detected in fusion protein overlays of lysate samples from brain [31], the tissue in which MAGI-2 is most abundantly expressed. MAGI-2 has been found to enhance the rate of agonist-promoted internalization of the β_1AR [44], which is the opposite effect of PSD-95 on receptor internalization. Another β_1AR-interacting PDZ protein is the cyclic nucleotide Ras guanine nucleotide exchange factor (CNRasGEF) [47], also known as PDZ-GEF-1 [48], nRAP-GEP [49], and RA-GEF [50]. CNRasGEF is a widely expressed cyclic AMP-binding protein that also acts as a guanine nucleotide exchange factor (GEF) for the small G protein Ras. The β_1AR/CNRasGEF interaction was detected in specific searches for a $G\alpha_s$-coupled receptor that could associate with the PDZ domain of CNRasGEF. β_1AR was examined in these searches because it had already been shown at that time to interact with PSD-95. CNRasGEF was found to not only associate with β_1AR but to also robustly facilitate β_1AR activation of Ras in cells [47]. The final PDZ protein that is known to interact with β_1AR is the GAIP-interacting protein (GIPC), which was pulled out of a heart library in a yeast two-hybrid screen with the β_1AR-CT [51]. GIPC is a widely expressed protein that possesses a single PDZ domain and is known to associate with GAIP, a known regulator of G protein signaling (RGS) [52]. The β_1AR/GIPC interaction was not found to have any effect on receptor internalization, but overexpression of GIPC was found to result in a potent attenuation of β_1AR-mediated extracellular signal-regulated kinase (ERK) activation [51].

Interestingly, all four of the β_1AR-interacting PDZ proteins have been found to recognize binding determinants within the last few amino acids of the β_1AR. However, the determinants that they recognize are not the same. For PSD-95, MAGI-2, and CNRasGEF, the terminal Val of the E-S-K-V motif is the most critical residue for association with β_1AR [37,44,47]. Conversely, mutation of this residue to alanine has little effect on the binding of GIPC, for which the Ser residue at the −2 position appears to be the most important determinant of the interaction [51]. Removal of the E-S-K-V motif from the β_1AR has been shown to result in profound alterations in receptor signaling and trafficking in cardiac myocytes lacking endogenous β_1AR [4], revealing a crucial role for this short motif in regulating β_1AR function in a cell type where the receptor is normally expressed.

It might seem superfluous for the last few amino acids of the β_1AR-CT to be able to bind to four different PDZ proteins. However, it is important to point out that these four proteins exhibit distinct patterns of expression across bodily tissues. Thus, β_1AR probably interacts with different PDZ partners in different tissue types. PSD-95 and MAGI-2 are both found almost exclusively in the brain, for example, and furthermore their expression levels within the brain differ markedly across different regions [38,46]. Meanwhile, CNRasGEF and GIPC exhibit radically different patterns of

expression across various tissues and, importantly, are both found in the heart [48,52], a tissue that possesses high levels of β_1AR but no PSD-95 or MAGI-2. It is well known that β_1AR exhibits differential behavior in different cellular contexts, with striking tissue-specific differences in the rate of receptor internalization being especially noteworthy [28,34,53,54]. It is likely that such differences in β_1AR behavior in distinct cells can be explained in large part by the differential expression of β_1AR-interacting proteins that regulate receptor function and trafficking.

It is likely that β_1AR/PDZ interactions are highly regulated in cells. There are several ways in which this regulation may occur: (i) competition, (ii) synergism, and (iii) post-translational modification. Competition might be expected to occur if a cell expresses more than one of the β_1AR-interacting PDZ proteins. The relative binding affinities of the β_1AR-CT for each of the PDZ proteins and the relative cellular concentrations of the various PDZ proteins would probably be the key factors in determining which protein would be dominant in the regulation of β_1AR function in any given cell. Conversely, in cases where the PDZ proteins are capable of interacting with each other, they may act synergistically to regulate receptor activity. For example, it has been shown that PSD-95 and MAGI-2 can directly associate [55], and it has also been shown that CNRasGEF and MAGI-2 can interact [49]. Thus, three of the four known β_1AR-interacting PDZ proteins have the potential to form a complex in cells and thereby work in concert to regulate β_1AR function, an interesting possibility that has not yet been explored experimentally. Finally, β_1AR/PDZ interactions may be reversibly regulated by post-translational modifications, such as protein phosphorylation. For example, it has been shown that GRK5-mediated phosphorylation of the Ser residue at -2 on the β_1AR-CT disrupts the receptor's association with PSD-95 [56]. Phosphorylation of this residue would also be expected to disrupt β_1AR interactions with the receptor's other PDZ partners, given that the Ser at the -2 position is critical for all of the known β_1AR/PDZ interactions. It is interesting to consider that GRK-mediated phosphorylation is capable of significantly rearranging β_1AR associations with cytoplasmic proteins, since GRK phosphorylation promotes receptor interactions with β-arrestins 9 while simultaneously disrupting β_1AR interactions with PDZ proteins [56].

3. β_2-Adrenergic receptor-interacting proteins

The first protein that was found to interact specifically with β_2AR but not with other βAR subtypes was the Na^+/H^+ exchanger regulatory factor 1 (NHERF-1) [57,58]. The β_2AR/NHERF-1 interaction was first detected in studies aimed at purifying β_2AR-CT-interacting proteins from kidney tissue lysates. NHERF-1 was originally identified as the protein co-factor necessary for inhibition of the Na^+/H^+ exchanger 3 (NHE3) by protein phosphorylation [59] and was also independently cloned as an ezrin-binding phosphoprotein of 50 kDa (EBP50) [60]. A closely related protein, called NHERF-2 and additionally referred to as E3KARP [61] and SIP-1 [62], also has been found to associate with the β_2AR-CT [58]. Both NHERF-1 and NHERF-2 contain two PDZ domains, the first of which mediates the interactions with the motif D-S-L-L at the end of the β_2AR-CT [57,58]. The fact that this motif is not conserved in the other βAR subtypes explains why the NHERF proteins interact selectively with β_2AR.

The physiological significance of the β_2AR/NHERF interaction has been elucidated in terms of both (i) how the receptor can regulate the cellular activity of the NHERF proteins and (ii) how the NHERF proteins can regulate the cellular activity of β_2AR. Concerning the receptor's regulation of the NHERF proteins, it has been known for at least two decades that β_2AR stimulation in the kidney exerts a paradoxical regulation of NHE3 activity: raising cyclic AMP levels typically *inhibits* NHE3 activity, but β_2AR stimulation *potentiates* NHE3 activity despite raising cyclic AMP levels [63,64]. It has been shown that mutation of β_2AR to block NHERF association switches β_2AR regulation of NHE3 from potentiation to inhibition, indicating that the ability of β_2AR to couple to the NHERF proteins in an agonist-promoted fashion is important for β_2AR-mediated regulation of NHE3 [57]. Cellular association of β_2AR with the NHERF proteins can also allow for β_2AR-mediated regulation of platelet-derived growth factor receptor (PDGFR) activity, since the NHERF proteins are potent regulators of PDGFR function [65]. In terms of NHERF regulation of β_2AR function, it has been shown that wild-type β_2AR normally recycles to the plasma membrane following agonist-promoted internalization, but mutation of the NHERF-binding motif results in the shunting of β_2AR to lysosomes and ultimately to receptor degradation following internalization [66]. Overexpression of the first PDZ domain of NHERF-1, which disrupts the association of β_2AR with endogenous NHERF proteins, alters β_2AR trafficking in a fashion that is similar to the effect of mutation of the receptor's NHERF-binding motif [66]. These findings reveal that the NHERF proteins play a pivotal role in directing β_2AR endocytic sorting.

Another protein that associates with the distal region of the β_2AR-CT is the *N*-ethylmaleimide-sensitive factor (NSF) [67]. This interaction was identified in yeast two-hybrid screens using the β_2AR-CT as bait, and was found to be dependent upon the same D-S-L-L motif as the β_2AR/NHERF interaction. However, NSF does not contain PDZ domains, and the precise amino acids that it recognizes on the β_2AR-CT are distinct from those recognized by the NHERF proteins. For example, the Leu residue at the -1 position is critically necessary for NSF binding [67] but does not play any role in NHERF binding [57,58]. Interestingly, mutation of this Leu at the -1 position to block NSF binding has been shown to result in the creation of a mutant β_2AR that is deficient in its ability to recycle to the plasma membrane following agonist-promoted internaliza-

tion [67]. This effect is similar to that observed following mutation of the terminal Leu residue [66], a mutation that disrupts both NSF and NHERF interactions with β_2AR. These data indicate that NSF plays a key role in controlling β_2AR trafficking in cells, and furthermore suggest that the mechanism by which the NHERF proteins regulate β_2AR endocytic sorting may be via competition with NSF for binding to the distal portion of the β_2AR-CT.

As mentioned earlier, a major pathway through which all of the βAR subtypes exert their cellular effects is via increasing cyclic AMP levels and thereby activating PKA. The well-known functional connection between βARs and PKA has inspired several groups to explore potential interactions between β_2AR and various PKA-associated anchoring proteins (AKAPs). Two AKAPs have been found to associate with β_2AR: AKAP250 (gravin) [68–70] and AKAP79 [71,72]. β_2AR association with these AKAPs promotes receptor phosphorylation [71,72], agonist-promoted internalization [69,71,72], and resensitization [68,69]. The molecular determinants of these interactions have not been finely mapped in terms of the specific amino acids involved on either β_2AR or the AKAPs, and therefore it is not known at present if these interactions are specific to β_2AR or rather if they are general to all βAR subtypes and/or other GPCRs.

There are a handful of other proteins that have been reported to interact with the β_2AR-CT: the eukaryotic initiation factor 2B (eIF-2B) [73], the adaptor protein Grb2 [74], and the tyrosine kinase Src [75,76]. For all of these proteins, as with the AKAPs, it is not known if the interactions are specific to β_2AR or general to all βAR subtypes. The interaction of β_2AR with eIF-2B was originally detected in an unbiased yeast two-hybrid screen, and overexpression of eIF-2B in cells was found to modestly promote β_2AR-mediated stimulation of adenylyl cyclase (∼15% increase) [73]. As with the interaction between endophilin-1 and β_1AR, however, elucidation of the true physiological significance of the eIF-2B/β_2AR interaction may depend on future insights into the various cellular roles of eIF-2B. Grb2 and Src have been shown to interact with the motif surrounding Tyr350 on the β_2AR-CT, and disruption of these interactions via mutation of Tyr350 has been found to promote β_2AR internalization and desensitization [74,75]. These interactions were not pulled out in a screen, but rather were specifically examined due to the fact that Grb2 and Src contain SH2 domains, which are modular domains known to interact with motifs on target proteins containing phosphotyrosine residues [77]. Since β_2AR was found to be phosphorylated on Tyr350 following cellular stimulation with insulin [78], it was hypothesized that the receptor might interact with SH2 domain-containing proteins, such as Grb2 and Src. The relative affinities of β_2AR for these SH2 proteins versus other SH2 proteins have not yet been explored, though, nor is it known whether these interactions can occur for other βAR subtypes (except in the case of β_3AR/Src, as discussed below).

4. β_3-Adrenergic receptor-interacting proteins

Since β_3AR was cloned several years after the other βAR subtypes and has a much more restricted tissue distribution [3], less is currently known about β_3AR interactions with cytoplasmic proteins than is known about the cellular partners of β_1AR and β_2AR. Presently, the only cytoplasmic protein other than G proteins that has been found to associate with β_3AR is the tyrosine kinase Src [79]. The SH3 domain of Src interacts with proline-rich sequences in the third intracellular loop and carboxyl-terminus of β_3AR, and this interaction has been shown to be critical for β_3AR-mediated MAP kinase activation in cells [79]. The future elucidation of other β_3AR-interacting proteins may shed light on the regulation of β_3AR signaling and trafficking in adipose tissue, where β_3AR plays a key role in the sympathetic regulation of lipid metabolism [13].

5. β-Adrenergic receptor dimerization with other receptors

GPCRs have traditionally been thought to act as monomers in the plasma membrane, but a large amount of evidence has emerged over the past decade to suggest that many GPCRs dimerize as part of their normal function [80]. One of the first GPCRs that was convincingly shown to be capable of homodimerization was the β_2AR, as revealed via both co-immunoprecipitation [81] and bioluminescence resonance energy transfer (BRET) [82,83]. β_1AR has also been shown to be capable of homodimerization [44,84,85]. Moreover, β_1AR and β_2AR have been found to be capable of robust heterodimerization [85,86], which may facilitate the cross-regulation of receptor internalization and signaling between these two receptor subtypes [85].

In addition to receptor–receptor interactions within the βAR family, βARs have also been found to heterodimerize with other GPCRs. For example, β_2AR has been found to heterodimerize with the δ-opioid receptor (δOR) [83,87,88]. There is no evidence that this interaction alters the receptors' pharmacological properties, but the association has been shown to allow for cross-internalization between agonist-stimulated β_2AR and δOR [87]. Similarly, both β_1AR and β_2AR have been found to heterodimerize with the α_{2A}-adrenergic receptor (α_{2A}AR), with the primary functional consequence also being receptor co-internalization upon agonist stimulation [89]. These receptor–receptor interactions are presumed to be direct, and may allow for forms of receptor cross-regulation that are distinct from the traditional heterologous desensitization pathways that are known to result from βAR activation of PKA [14].

6. β-Adrenergic receptor interactions with ion channels

β-Adrenergic receptors are known to mediate many of their physiological effects via the regulation of various ion

channels, notably calcium channels [90], potassium channels [91], and NMDA-type glutamate receptor channels [39–41]. The formation of physical complexes between βARs and ion channels might be expected to facilitate receptor-mediated channel regulation via G protein-dependent mechanisms and to also potentially allow for more direct forms of regulation. Receptor/channel complexes can be formed through the actions of receptor-associated scaffold proteins, as in the case mentioned above for β_1AR association with NMDA-type glutamate receptor channels via PSD-95 [37]. βARs may also associate directly with certain channels, as described for the interaction of β_2AR with the L-type calcium channel $Ca_v1.2$ [92]. The specificity or generality of direct βAR/channel interactions has not yet been widely explored, and may be an area of significant future research interest.

7. Summary and perspectives

The three β-adrenergic receptor subtypes all couple efficiently to G proteins but associate differentially with other proteins. The various βAR-associated proteins exhibit distinct patterns of tissue localization, which may underlie the differential behavior of βAR subtypes that is known to occur in different cellular contexts. Since βARs are extremely common targets for therapeutic pharmaceuticals, the disruption of βAR interactions with cytoplasmic regulatory proteins may represent a useful approach for the development of future therapeutics, since drugs acting in this way might potentially have effects that are more tissue-specific than drugs acting directly on the receptors themselves. To lay the foundation for the potential development of such therapeutics, however, it is necessary to first characterize the full set of βAR-interacting proteins and the impact of these proteins on receptor physiology. As summarized here, there have been many exciting findings in this area over the past several years, with more insights likely to come in the near future.

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