EDITORIAL COMMENT

Dissecting Beta-Adrenergic Receptors

The Sum of Many Parts*

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Although the 7-transmembrane-spanning G-protein-coupled beta$_1$-adrenergic receptor (β_1AR) has been studied extensively, it seems that around every corner, another regulatory and/or signaling mechanism comes to light. Molecular cloning of beta-adrenergic receptors (βARs) has facilitated ascribing molecular functionality to multiple regions, most notably the intracellular loops and the carboxy tails.[1,2] More specifically, a vast number of studies have determined that the carboxy terminus of βARs contains several regulatory phosphorylation sites for the protein kinase A (PKA), protein kinase C, and beta-adrenergic receptor kinases (G-protein-coupled receptor kinases),[3,4] resulting in desensitization—that is, uncoupling from or differential coupling to—G-proteins and their cognate downstream signaling pathways (PKA, calcium calmodulin-dependent protein kinase II, mitogen-activated protein kinase [MAPK], protein kinase B, and so on). Further, phosphorylation of the carboxy terminus is associated with changes in receptor trafficking; internalization; and ultimately, either receptor degradation or resensitization by lysosomal phosphatases and recycling to the sarcolemmal membrane. Additional studies have mapped out the regions of intracellular loop 3 interacting with G-proteins Gαs and GαI and their cognate signaling pathways. Finally, numerous studies, both biochemical and structural, have elucidated in detail the binding sites of βAR ligands and afforded, via examination of structural activity relationships, the ability to devise molecules with novel allosteric or biased signaling properties. Contemporaneously, numerous biochemical and structural investigations have elucidated the overall topology of βARs, including the finding that the extracellular N-terminus is glycosylated.[5] These early studies demonstrated that βAR glycosylation plays important roles in receptor degradation[6] and, interestingly, in receptor dimerization.[7] In contrast to O-linked glycosylation, discussed later, modification of the single N-linked glycosylation β_1AR site at Asn^{15} does not seem to modify agonist-induced internalization but does appear to influence β_1AR homodimerization and heterodimerization.[7]

In the first description of β_1AR N-terminal fragmentation,[8] Western blot analysis indicated bands of ~relative molecular mass 69k and 54k. This finding led to speculation that βARs could undergo metalloproteinase-dependent proteolytic cleavage. Each of these peptide bands could be further reduced in size by treatment with both N- and O-deglycosylating enzymes.[8] It is important to note, however, that the 2 dominant cardiac and vascular βARs, β_1AR and β_2AR, are both glycosylated but do not share N-terminal peptide sequence homology.[9] Thus, the potential regulatory role(s) of glycosylation-mediated N-terminal cleavage of the β_1AR may not translate functionally to the β_2ARs.

In this issue of *JACC: Basic to Translational Science*, Zhu and Steinberg[10] build on an extensive record of the group's investigation of βAR glycosylation and its relevance to cardiac signaling competency. In the Steinberg group's first major foray into βAR glycosylation, Park et al[9] determined that β_1AR O-glycosylation at Ser^{37}/Ser^{41} inhibited N-terminal cleavage. Further, they demonstrated that the full-length glycosylated β_1AR as well as a truncated O-glycosylation-defective mutation were both competent to signal via the PKA and MAPK

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pathways. However, the N-terminal-truncated mutation (Ad-δ2-52-β1-AR) demonstrated a bias toward the cyclic adenosine monophosphate (cAMP)/PKA pathway with a diminution in the phosphorylation of MAPK. Interestingly, a polymorphic variation of β_1AR Ser49Gly known to have differential signaling properties and a potential site of O-linked glycosylation does not appear to substantially affect the overall β_1AR glycosylation profile.[9]

As a follow-on, an elegant study by Zhu and Steinberg[11] determined that major sites of β_1AR N-terminal cleavage occur at $R^{31}{\downarrow}L^{32}$ and $S^{41}{\downarrow}L^{42}$ by the matrix metallopeptidase ADAM17 and are regulated by the presence of O-glycosylation at S^{37} and S^{41}. In that same report, it was also demonstrated that oxidative stress, secondary to H_2O_2 exposure, led to increased ADAM17-mediated N-terminal cleavage of the full-length wild-type β_1AR, the effect being abrogated by the use of ADAM10/17 inhibitors. This is clearly of physiologic relevance in the context of myocardial ischemia and reperfusion injury.

Most recently, Zhu and Steinberg[10] turned their attention to β_1AR cleavage and regulation by elastase. This is particularly important given the role of inflammatory processes concomitant with myocardial ischemia and heart failure. Elastase, a serine protease, is more commonly known to be secreted by the pancreas. However, it is also released by activated neutrophils at the sites of cardiac injury. Interestingly, the site of elastase-mediated β_1AR cleavage is unique, differing from those described previously for ADAM17 at $R^{31}{\downarrow}L^{32}$, $S^{41}{\downarrow}L^{42}$, and $P^{52}{\downarrow}L^{53}$. More specifically, a sialylated O-glycan modification of T^{28} appears to prevent elastase-mediated N-terminal cleavage. In an elegant permutation, the authors show that a T28A substitution that is not O-glycan modified "renders 'cleavage-resistant' β_1AR-31/52 and β_1AR-31/41/52 mutants elastase-sensitive." However, the novel T28-protected cleavage site, like those previously described, is cleavable by ADAM17 because it is blocked by an ADAM17-selective inhibitor.

Importantly—and in contrast to the previously described ADAM-mediated β_1AR N-terminal truncations—the elastase N-terminal truncation is markedly less sensitive to isoproterenol-mediated cAMP accumulation. In cardiomyocytes, elastase was also shown to cleave the β_1AR at a second site not located in the N-terminus, tentatively mapped to extracellular loop 2. Cleavage there led to a functionally incompetent receptor, at least as assessed by cAMP accumulation in response to isoproterenol.

The fact that the human βARs undergo multiple posttranslational modifications—including phosphorylation by multiple kinases and lipid modification, that is, palmitoylation, S-nitrosylation, and so on—has broad ramifications for receptor expression, localization, and differential signaling. The combinatorial permutations are vast and are likely influenced further by cell type. In a therapeutic era that is evolving beyond the current standards of heart failure therapy, refined definitions of βAR modifications may lead to novel compounds. For example, the presence or absence of a specific N-terminus truncation, if able to be regulated, might be seen as the molecular equivalent of a "biased ligand" with differential activation of signaling pathways—for example, PKA vs MAPK and protein kinase B—and their downstream targets. In the current context, a further understanding of the regulatory basis of its proteolytic cleavage could lead to new therapeutic modalities with enhanced salutary βAR signaling properties. To be determined.

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