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BETA$_1$-ADRENERGIC RECEPTOR REGULATION REVISITED; THE ROLE OF THE EXTRACELLULAR N-TERMINUS

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Abstract

The actions and regulation of cardiomyocyte β_1ARs differ in several respects from the properties described for the prototypical β_2AR subtype; a mechanism to explain the unique properties of the β_1AR subtype has never been obvious. This viewpoint summarizes recent studies that identify a novel signaling paradigm for the β_1AR, implicating the N-terminus as a molecular determinant of β_1AR responsiveness.

Keywords

Cell Signaling/Signal Transduction; heart failure; oxidant stress

SCIENTIFIC METHOD

> It is a capital mistake to theorize before one has data. Insensibly one begins to twist the facts to suit theories, instead of theories to suit the facts.
>
> SIR ARTHUR CONAN DOYLE
>
> It is also a good rule not to put too much confidence in experimental results until they have been confirmed by theory.
>
> SIR ARTHUR EDDINGTON
>
> First get your facts; then you can distort them at your leisure.
>
> MARK TWAIN

Beta-adrenergic receptors (βARs) are among the most intensively studied members of the G protein-coupled receptor (GPCR) superfamily. These receptors, which transduce signals derived from catecholamine binding to a cellular response, have garnered considerable interest as therapeutic targets because of their key roles in the physiologic control of cardiovascular performance as well as the pathogenesis of cardiac arrhythmias, ventricular remodeling, and the evolution of heart failure (HF). Breakthroughs in receptor biology, culminating most recently with the development of strategies to obtain high-resolution GPCR structures, have lead to a detailed understanding of the βAR's two core functions -

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namely ligand binding to a transmembrane ligand-binding pocket and G protein- or β-arrestin-induced cellular activation [1]. These studies also have exposed desensitization mechanisms that provide a critical homeostatic control to avoid excessive/unrelenting GPCR activation. However, it is important to note that much of our current concepts regarding the mechanisms that activate and/or regulate βARs come from studies of the β_2AR subtype, the first hormone-activated GPCR to be cloned and structurally characterized. Based upon early studies that identified essentially equivalent signaling properties for β_1ARs and β_2ARs in certain model cell types as well as evidence that various aspects of the structural architecture, signaling mechanisms, and regulatory machinery are highly conserved across the GPCR superfamily, the literature has generally viewed the β_2AR as a useful surrogate for the β_1AR subtype. However, this assumption ignores literature that identifies discrepancies between the properties of β_1 vs. β_2ARs. My laboratory has struggled for some time with the nagging suspicion that some aspects of the actions and regulation of the cardiomyocyte β_1AR may not adhere to conventional models described for the prototypical β_2AR subtype.

Studies in transgenic models provided some of the earliest evidence for functional divergence between β_1 and β_2ARs showing that high levels of transgenic β_2AR overexpression are well tolerated, whereas even relatively low levels of transgenic β_1AR overexpression result in maladaptive cardiac remodeling and HF [2, 3]. Studies in cell-based models also established that chronic catecholamine stimulation leads to cardiomyocyte apoptosis through the activation of β_1ARs, whereas β_2AR stimulation may in fact be cardioprotective [4].

Immunoblotting studies performed to validate the β_1AR knockout mouse model identified another distinctive feature of β_1ARs that defied obvious explanation. These Western blots identified molecular heterogeneity for the β_1AR subtype that was not evident in immunoblotting studies of β_2ARs [5]. Recent studies from my laboratory establish that β_1ARs are susceptible to N-terminal cleavage [6], similar to the processing mechanism originally described for the turkey βAR – the avian homologue of the mammalian β_1AR subtype. The observation that β_1ARs are expressed as both a full-length protein and a smaller N-terminally truncated species raises the first obvious question: ***What controls the generation of the distinct molecular forms of the β_1AR? Do distinct β_1AR species signal differently?***

Other studies exposed differences in susceptibility to agonist-induced desensitization/internalization. The classical paradigm holds that agonist-activated βARs are phosphorylated by G protein-coupled receptor kinases (GRKs), leading to the recruitment of β-arrestin, and receptor desensitization/downregulation. Head-to-head comparisons of β_1 versus β_2ARs indicate that agonist-induced phosphorylation, internalization/sequestration, and/or down-regulation is blunted for the β_1AR subtype, compared to the β_2AR [7–10]. Sequence comparisons provide a likely explanation; β_1- and β_2ARs share considerable sequence homology in their transmembrane ligand-binding pockets, but other regions of these receptors are more divergent. In particular, sites on the intracellular surface of the β_2AR that serve as substrates for GRK phosphorylation and/or docking sites for β-arrestin are not conserved in β_1ARs. Therefore, the observation that HF (a condition characterized by elevated catecholamine levels) leads to selective desensitization/downregulation of the β_1AR

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subtype and is not accompanied by a commensurate loss of β_2ARs seems paradoxical [11]; the prediction based on cell-based studies is that chronic catecholamine-induced, GRK/β-arrestin-dependent desensitization would lead to a decrease in β_2ARs, and that the β_1AR subtype would be relatively spared. Hence, question #2: ***What mechanism underlies the HF-induced decrease in β_1ARs?***

We recently made progress toward answering both of these questions through studies of the β_1AR N-terminus, the relatively short/unstructured extracellular portion of the receptor that differs in length, sequence, and post-translational processing from the β_2AR N-terminus. We showed that the β_1AR N-terminus contains two O-linked glycosylation sites at Ser^{37} and Ser^{41} and that O-glycosylation is required for full-length β_1AR expression; β_1ARs accumulate as N-terminally truncated species under conditions that prevent β_1AR O-glycosylation ([6] as if the mucin-like O-glycans attached to the N-terminus act as a barrier to prevent protease access and cleavage at an adjacent site, Figure 1). An O-glycosylation-regulated N-terminal cleavage mechanism that gives rise to distinct molecular forms of the β_1AR provides the first credible explanation for the molecular heterogeneity displayed by native β_1ARs in various cardiac preparations. It also emphasizes that previous literature using immunblotting or immunohistochemical methods to track β_1AR expression and/or localization using antibodies to a transgene's N-terminal tag should be interpreted with caution, since these techniques do not capture N-terminally truncated β_1AR species that lack the N-terminal epitope tag.

Does β_1AR molecular heterogeneity matter?

We recently reported that N-terminally truncated β_1ARs remain signaling competent, but N-terminal truncation alters signaling bias to the cAMP/PKA vs. ERK pathways [6]. The notion that an O-glycosylation-regulated N-terminal cleavage mechanism can regulate signaling by a GPCR is quite novel and is predicted to have pathophysiological significance since O-linked glycan structures are highly regulated during developmental and in response to inflammatory stimuli, metabolic disorders, and abnormalities in cell growth; alterations in glycan structures are detected in cardiac hypertrophy [12].

How does the N-terminus function as a molecular determinant of β_1AR responsiveness?

In theory, the N-terminus might function as an allosteric regulator of the β_1AR structure itself, a regulator of β_1AR trafficking to subcellular compartments, and/or a regulator of β_1AR coupling to signaling partners and downstream effector responses. These mechanisms are not mutually exclusive and are the focus of ongoing studies.

What is the mechanism underlying the HF-induced decrease in β_1AR density?

In an effort to identify stimuli that might regulate β_1AR cleavage, we stumbled upon evidence that oxidative stress (a stimulus that contributes to the pathogenesis of HF) leads to a selective decrease in cardiomyocyte β_1AR expression that is not associated with a change in the abundance of the β_2AR subtype [13].

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Carvedilol to the rescue!

Attempts to identify a mechanism for (and strategies to prevent) the ROS-dependent decrease in β_1AR expression lead to two intriguing observations regarding the pharmacologic properties of carvedilol. First, our studies showed that the redox-dependent decrease in β_1AR expression is prevented by carvedilol (but not a panel of other βAR ligands) and that this cannot be attributed to carvedilol's ancillary anti-oxidant properties [13]. While the molecular mechanism underlying carvedilol's ability to protect β_1ARs from redox-inactivation remains uncertain, it is worth noting that carvedilol contains a bulky aromatic amine substitution (not present in most other adrenergic ligands) that makes unique contacts with an extended β_1AR ligand binding pocket that includes the redox-sensitive cysteines in extracellular loop 2 [14]. In theory, carvedilol might protect β_1ARs from redox-inactivation either by shielding these cysteines from redox-inactivation or producing a conformational rearrangement of the extracellular surface so as to bury the redox-sensitive disulfide bonds.

Carvedilol treatment also leads to the accumulation of N-terminally truncated β_1ARs that constitutively activate the AKT pathway and protect against doxorubicin-induced apoptosis (Figure 1 [13]). This second cardioprotective property for carvedilol (not shared by other βAR ligands) is predicted to have important clinical and therapeutic implications. First, it identifies a mechanism that presumably underlies the protective effects of prophylactic carvedilol against anthracycline-induced cardiotoxicity in the clinic. Second, these findings may be pertinent to the lingering controversy regarding the interpretation of the COMET trial results, which ascribed significant survival advantage to carvedilol over metoprolol in the treatment of HF. The unique pharmacologic action of carvedilol not shared with metoprolol or other βAR blockers identified in our studies is predicted to offer to meaningful cardioprotection.

This viewpoint presents a revised model of β_1AR action and regulation that challenges conventional dogma in that it identifies a novel role for the β_1AR N-terminus as a molecular determinant of catecholamine responsiveness. We recognize (and are open to the fact that) some aspects of our model might not withstand the scrutiny of future research. Nevertheless, these newer studies set the stage for future research that examines factors (and proteases) that control β_1AR N-terminal cleavage and the role of glycosylation-defective/N-terminally truncated β_1ARs in the evolution of catecholamine-induced pathologic cardiac remodeling *in vivo.* Our hope is that this line of research will reveal novel strategies targeted to processing events localized to the β_1AR N-terminus that might ultimately be used for therapeutic advantage.

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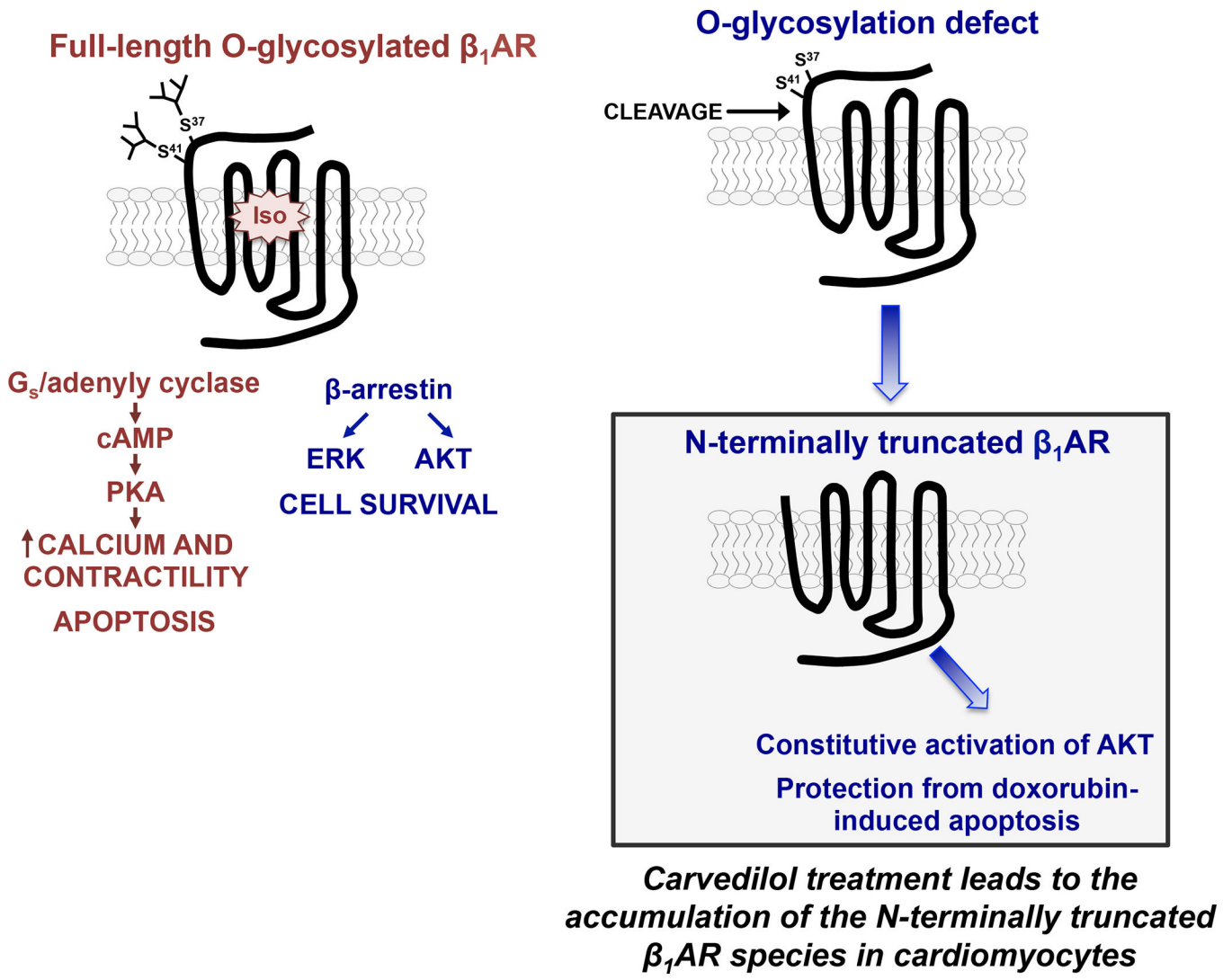

Figure 1:
β_1AR N-terminal processing (see text).