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β_3-Adrenergic Receptor Subtype Signaling In Senescent Heart

Nitric Oxide Intoxication or "Endogenous" β Blockade for Protection?

COMPLEX biochemical, pharmacologic, and physiologic changes occur with advancing age in the myocardium. These changes may be subtle and virtually undetectable under resting steady state conditions, but become prominent under stressful stimulation. In this issue of ANESTHESIOLOGY, Birenbaum *et al.*[1] provide evidence for impaired inotropic response (approximately 30%) to β-adrenergic stimulation in aged *versus* young rat hearts. Apart from β_1-adreneric receptor (AR) down-regulation, they further demonstrate that increased formation of nitric oxide deriving from neuronal nitric oxide synthase (nNOS) *via* the activated β_3-AR subtype signaling pathway is responsible for the reduced inotropy in senescent hearts.

Since the late 1980s, it is known that three β-AR subtypes, namely the β_1-, β_2-, and β_3-AR, participate in the regulation of cardiovascular function. Expression of β_3-ARs in human hearts was first reported in 1996 by Gauthier *et al.*[2] The β_3-AR shares only approximately 50% homology with $\beta_{1/2}$-ARs and is particular in certain aspects (table 1). First, in contrast to $\beta_{1/2}$-ARs (monoexonic genes), the gene encoding this receptor on chromosome 8 contains two exons and one intron enabling alternative splicing and formation of two different receptor isoforms with different pharmacologic properties.[3] Second, the β_3-AR lacks phosphorylation sites for G protein–coupled receptor kinase and protein kinase A in the cytoplasmic C-terminus tail, and thus is resistant to catecholamine-induced desensitization.[4] Third, besides the strong lusitropic effects, the majority of studies suggest inhibition of cardiac contractility after β_3-AR stimulation. Hence, in the presence of labetalol or prazosin plus nadolol, *i.e.*, α- plus $\beta_{1/2}$-AR blockade, norepinephrine becomes cardiodepressive.[5] In cardiomyocytes (fig. 1), β_3-ARs activate, through a pertussis toxin–sensitive G_i protein–coupled mechanism, endothelial nitric oxide synthase (eNOS or NOS3), located between sarcolemmal and T-tubular caveolae, and nNOS (or NOS1), located in the sarcoplasmic reticulum (or sarcolemma in the failing heart), and exert nitric oxide–mediated negative inotropic effects *via* the guanylyl cyclase–cyclic guanosine monophosphate pathway on L-type Ca^{2+} channels and on myofilaments.[5–8] Nitric oxide further inhibits contractility by S-nitrosylation of key proteins of the respiratory chain,[9] energy metabolism,[10] contractile apparatus, and Ca^{2+} handling, and it potentiates the inhibitory effect of the Gi protein on protein kinase A by activating phosphodiesterase II,[11] thereby reducing the second messenger cyclic adenosine monophosphate of the Gs-coupled $\beta_{1/2}$-ARs. A last feature of the β_3-AR is its low expression in healthy myocardium with a preference in left ventricular tissue, while its expression is reported to increase by two to three times in various pathologic conditions including sepsis,[12] diabetes,[13] and heart failure[14] and, as shown in the current study by Birenbaum *et al.*,[1] during the aging process.

In perioperative medicine, the sympathetic nervous system plays an important role in the pathogenesis of cardiovascular complications.[15] Therefore, gaining control over the adrenergic activity represents a core task in anesthetic practice.[16,17] On the other side, sustained activation of the sympathetic nervous system with enhanced β-AR signaling plays an important role in cardiovascular aging by promoting inflammation, oxidation and nitrosylation of key proteins, and cell death.[18] Consistent with the free radical theory of aging, previous studies showed increased formation of reactive oxygen and nitrogen species in aged myocardium, mainly generated by mitochondria and nitric oxide synthase (NOS) isoforms.[19] Nitric oxide is produced by nearly all cell types in the heart, and is synthesized from L-arginine by the catalytic reaction of the three different highly compartmentalized isoforms of nitric oxide synthase (neuronal or type 1 NOS [nNOS or NOS1], inducible or type 2 NOS [iNOS or NOS2], and endothelial or type 3 NOS [eNOS or NOS3]).[20] In the aged heart, iNOS and nNOS, mainly found in cardiomyocytes and autonomic nerves, are up-regulated, whereas eNOS, primarily expressed in endothelial cells (but also myocytes), is down-regulated.[21,22] In the adult heart, nitric oxide regulates cardiac biology by affecting energy metabolism, substrate utilization, apoptosis, hypertrophy, regeneration, and preconditioning.[23] However, its biologic effects are often "double-edged," with a variety of opposing effects depending on the dose and site of action within the cell. Li *et al.*[24]

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Table 1. β-Adrenergic Receptor Subtype Characteristics

Receptor subtype Main signaling components *Location*	Effector cells	Predominant effects	Relative receptor density in left ventricle, %
β_1			70–80
Gs *Postsynaptic*	Cardiac myocytes	Increases heart rate Increases contractility Increases relaxation	
β_2			20–30
Gs/Gi *Presynaptic and postsynaptic*	Cardiac myocytes	Increases heart rate Increases contractility Increases relaxation	
	Peripheral and coronary vascular smooth muscle cells	Vasodilation	
	Bronchiolar smooth muscle cells	Bronchodilation	
β_3			<2
Gi/NO	Cardiac myocytes	Decreases heart rate Decreases contractility Increases relaxation	
Gs *Presynaptic and postsynaptic*	Peripheral and coronary vascular smooth muscle cells	Vasodilation	

Gi and Gs = inhibitory and stimulatory G-protein regulating adenylyl cyclase; NO = nitric oxide.
Based on references 8, 15, 34, and 35.

demonstrated the deleterious effects of nitric oxide derived from up-regulated iNOS in isolated perfused aged *versus* young rat hearts. These authors found increased nitric oxide and peroxynitrite formation deriving from up-regulated iNOS, which aggravated postischemic cardiac dysfunction and enlarged infarct size under β-AR stimulation with isoproterenol in aged *versus* young hearts. nNOS expression was also increased in aged hearts in that study, but its contribution to isoproterenol-induced postischemic damage was not investigated. Nonetheless, this study provides evidence that a phenotypic change of NOS isoforms in aged hearts may predispose to increased myocardial damage under sympathetic tone. As can be speculated from the results by Birenbaum *et al.*,[1] the observed detrimental effect in the study of Li *et al.*[24] may be well explained by upregulation of β_3-ARs. Augmented cardiac nitric oxide formation by β_3-AR signaling was also reported to be deleterious in sepsis, where the release of endogenous inflammatory mediators such as tumor necrosis factor α and interleukin 1β dramatically decrease the responsiveness of cardiomyocytes to catecholamines.[12] Interestingly, this refractoriness to catecholamines is absent in septic β_3-AR knockout mice.[12] In contrast to the toxic effects of β_3-AR signaling in certain conditions, in healthy adult hearts, β_3-AR signaling is necessary to counteract the proarrhythmogenic, and otherwise unchained chronotropic, dromotropic, and inotropic effects of $\beta_{1/2}$-ARs.[8] This phylogenetically highly conserved and protective negative feedback loop against catecholamine toxicity is important for a balanced and fine-tuned contractility.[8] Generally speaking, β_3-AR signaling acts as a countervailing "brake" against adrenergic overstimulation comparable with an "endogenous" β blockade. This concept is further supported by the fact that β_3-ARs maintain cardiac sympathovagal balance by reinforcing vagal tone. Beyond these benefits, but in contrast to pure β blockade, nitric oxide from β_3-AR signaling further exerts coronary and peripheral vasodilatation, and may have "pleiotropic" effects on cardiac stem cell biology[25] and the prevention of arteriosclerosis.[26] Indeed, the "pharmacologic profile" of β_3-AR signaling strongly reminds of the one observed with the novel third-generation β-blocker nebivolol, which promotes nitric oxide formation.[27] Research in the field of heart failure also supports the idea that β_3-AR signaling is beneficial rather than detrimental. In the failing heart, the β_3-AR response is preserved even under sustained activation of the adrenergic system and thus antagonizes catecholamine toxicity. However, this does not exclude that adverse effects do occur from β_3-AR–mediated negative inotropy, particularly during the progression of heart failure. Interestingly, β_3-AR knockout mice show increased SERCA2a expression and phospholamban phosphorylation resulting in enhanced SERCA2a activity.[28] Hence, one could speculate that β_3-AR blockade could play a salutary role in the therapy of heart failure, at least at later stages of the disease. Together, the biologic consequences of enhanced β_3-AR signaling range from "nitric oxide intoxication" on one side to protection comparable to some sort of "physiologic" or "endogenous" β-blockade on the other side. Although the study by Birenbaum *et al.*[1]

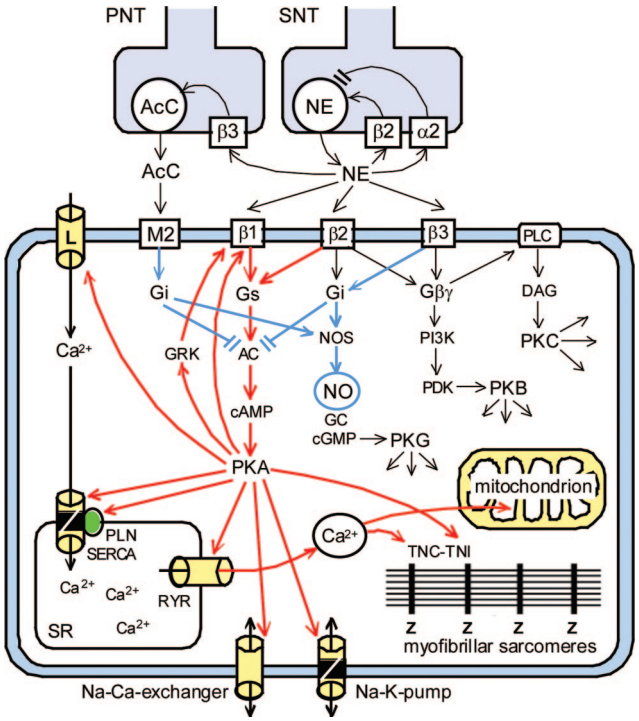

Fig. 1. Interrelation between β_3-adrenergic receptor (AR) and the major sympathetic and parasympathetic signaling pathways in cardiomyocytes. In the inotropic β_1- and β_2-AR signaling path (*red arrows*) protein kinase A (PKA) coordinates (1) positive inotropy by increasing intracellular Ca^{2+} (stimulation by phosphorylation of the L-type Ca^{2+} channel, of the sarcoplasmatic reticulum [SR] Ca^{2+} pump [SERCA] directly and indirectly by relieving its repression by phospholamban [PLN], and of the sarcoplasmatic reticulum Ca^{2+} release channel [RYR]); (2) positive lusitropy by removing cytoplasmic Ca^{2+} *via* the Na–Ca^{2+} exchanger and decreasing the affinity of Ca^{2+} binding to Ca^{2+} binding troponin subunit (TNC) by phosphorylation of inhibitory troponin subunit (TNI); (3) negative adrenergic feedback by desensitization of β_1-ARs (phosphorylation by PKA and G protein–coupled receptor kinase [GRK]); and (4) stimulation of adenosine triphosphate production in the mitochondria by Ca^{2+}. β_3-AR signaling (*blue arrows*) counterbalances the canonical β_1- and β_2-AR path *via* Gi and nitric oxide (NO) production as well as by enhancing the vagal tone by facilitation of acetylcholine (AcC) release from the parasympathetic nerve terminals acting through the muscarinic acetylcholine receptor 2 (M2). Norepinephrine (NE) release from the sympathetic nerve terminals exerts a second negative feedback loop. *Arrows* denote stimulation, and *blunted ends* denote inhibition. AC = adenylyl cyclase; DAG = diacylglycerol; GC = soluble guanylyl cyclase; Gi and Gs = inhibitory and stimulatory G-protein α subunits; L = L-type slow Ca^{2+}-channel; NOS = nitric oxide synthase; PDK = 3-phosphoinositide–dependent kinase; PI3K = phosphoinositide-3 kinase; PKA, PKB, PKC, and PKG = target-specific Ser/Thr protein kinases; PLC = phospholipase C; PNT = parasympathetic nerve terminal; SNT = sympathetic nerve terminal; Z = sarcomeric Z disc.

shows undesirable short-term effects of enhanced β_3-AR signaling on inotropy in aged hearts, the biologic (long-term) consequences of this pathway remain elusive.

A number of important questions need to be addressed in future studies before we can translate the findings of Birenbaum *et al.* to the clinical arena. First, based on genome-wide transcriptional profiling, the molecular mechanisms involved in reduced β-adrenergic responsiveness in the aged heart seem to be more complex and not just confined to a single signaling pathway. Accordingly, Dobson *et al.*[29] reported up-regulation of 19 transcripts involved in age-related antiadrenergic activity, including adenosine A_1, muscarinic M_3, and nicotinic β_3 acetylcholine receptors, and many more. Second, female tissue was reported to express less β_3-ARs,[30] and nitric oxide is differentially regulated in male and female hearts, as evidenced in nNOS knockout mice, where males develop more pronounced cardiac remodeling than females.[31] Therefore, sex-based differences may be of relevance in β_3-AR and nitric oxide signaling, specifically in aged hearts. Third, although the rat and human β_3-AR share 79% identity, there are pronounced heterogeneous pharmacologic profiles of β_3-AR agonists/antagonists between different species. Therefore, caution must be applied in extrapolating data obtained from rat hearts to human myocardium. Finally, β_3-ARs are also expressed in vessels. Namely, β_3-ARs are expressed on the endothelium of coronary microarteries,[32] but their role in ischemia–reperfusion is unknown, and it is unclear, to date, whether vascular β_3-ARs are similarly up-regulated during the aging process.

Modulation of β_3-AR signaling in the diseased heart in general and in the senescent heart in particular opens new therapeutic approaches. However, much research is required to fully understand the complexity of β-AR biology with its contrasting influences. New compounds with β_3-AR agonistic and/or antagonistic properties could help to better modulate and control the adrenergic system in the perioperative period. For example, in coronary artery bypass graft surgery patients, there is $\beta_{1/2}$-AR but not β_3-AR dysfunction after cardiopulmonary bypass, and β_3-AR stimulation could increase graft flow, decrease oxygen consumption, and thus protect the heart. However, these effects must be carefully balanced against the negative inotropy of β_3-AR signaling. Interestingly, the single nucleotide polymorphism of the β_3-AR at codon 64 from tryptophan to arginine was found to be associated with insulin resistance, diabetes, obesity, and hypertension.[8] Hence, patient studies investigating the impact of β-AR genomics on cardiac biology and clinical outcome might help to discern the therapeutic potential of β_3-AR signaling in the context of other important adrenergic polymorphisms[33] in human heart disease. Clearly, the findings by Birenbaum *et al.*[1] are important and will stimulate future research aiming at improving the cardiovascular management of elderly surgical at-risk patients. In this challenging endeavor, however, we need to expand our understanding of complex β-AR signaling beyond the platitude that "it is not by the gray of the hair that one knows the age of the heart" (Robert Bulwer-Lytton).

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