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Review Article

Different effects of prolonged β-adrenergic stimulation on heart and cerebral artery

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ABSTRACT

The aim of this review was to understand the effects of β-adrenergic stimulation on oxidative stress, structural remodeling, and functional alterations in the heart and cerebral artery. Diverse stimuli activate the sympathetic nervous system, leading to increased levels of catecholamines. Long-term overstimulation of the β-adrenergic receptor (βAR) in response to catecholamines causes cardiovascular diseases, including cardiac hypertrophy, stroke, coronary artery disease, and heart failure. Although catecholamines have identical sites of action in the heart and cerebral artery, the structural and functional modifications differentially activate intracellular signaling cascades. βAR-stimulation can increase oxidative stress in the heart and cerebral artery, but has also been shown to induce different cytoskeletal and functional modifications by modulating various components of the βAR signal transduction pathways. Stimulation of βAR leads to cardiac dysfunction due to an overload of intracellular Ca^{2+} in cardiomyocytes. However, this stimulation induces vascular dysfunction through disruption of actin cytoskeleton in vascular smooth muscle cells. Many studies have shown that excessive concentrations of catecholamines during stressful conditions can produce coronary spasms or arrhythmias by inducing Ca^{2+}-handling abnormalities and impairing energy production in mitochondria, In this article, we highlight the different fates caused by excessive oxidative stress and disruptions in the cytoskeletal proteome network in the heart and the cerebral artery in responsed to prolonged βAR-stimulation.

1. Introduction

Chronic increased sympathetic activation occurs in many situations, including obesity, sleep apnea, mental stress, and hypertension, promoting the development of cardiovascular diseases through sustained stimulation of adrenergic receptors.[1–4] These fatal cardiac events include cardiac hypertrophy, heart failure, and sudden cardiac death.[5–9] Elevated levels of catecholamines stimulate the α-adrenergic receptor and β-adrenergic receptor (βAR); however, most of the adverse cardiac effects associated with increased sympathetic tone on the heart have been believed to be caused mostly by

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stimulation of βAR in the heart. In fact, βAR blockade consistently improves cardiac function and survival in patients with heart failure.[10,11] By contrast, α-adrenergic receptor blockades is an effective antihypertensive approach, but may actually increase the risk of cardiovascular events, as shown in patients taking doxazosin in the ALLHAT (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial) trial.[12] Use of the βAR blocker metoprolol during the perioperative period in patients with non-cardiac diseases was associated with an increased the risk of strokes and death.[13] These studies suggest that sympatholytic agents are unlikely to be accepted as a common regimen for the treatment of both the heart and the vasculature simultaneously. Based on these findings, over-stimulation of βAR appears to have different effects on the heart and cerebral artery. Therefore, the focus of this review is to compare cardiac and vascular, effects of beta AR stimulation and effects on related signal transduction processes.

2. Effect of prolonged βAR stimulation on the heart

Long-term βAR activation by various stressors induces serious myocardial damages, including cardiac hypertrophy, necrosis/apoptosis, and fibrosis.[7,9,14–16] Cardiac hypertrophy is an independent cause of heart failure and major cause of morbidity and mortality throughout the world; thus, research and clinical interventions for cardiac hypertrophy have been extensively studied.[17–19]

Once cardiac hypertrophy develops, it progresses to heart failure.[17] The underlying mechanisms associated with βAR overstimulation have been studied *in vivo* in heart tissue using isoproterenol (ISO)-treated models and *in vitro* in cultured cardiomyocytes. This βAR overstimulation represents an important hallmark of pathologic cardiac hypertrophy.[15,20–24]

ISO treatment increases oxidative stress, protein synthesis, proto-oncogene expression, and stimulation of mitogen-activated protein kinases. These events are caused by altered of electrical and mechanical capabilities that induce three modes of cell death: necrosis, apoptosis, and autophagy (see Table 1).

Furthermore, ISO treatment alters related signal transduction pathways. In the normal heart, βAR activation stimulates adenylyl cyclase activity via Gs protein-coupled receptors, which leads to the formation of cAMP. Increased cAMP elevates intracellular concentrations of Ca^{2+}, which activates protein kinase A (PKA)-mediated phosphorylation of different Ca^{2+}-handling proteins, producing positive inotropic effects in the heart. However, long-term ISO stimulation results in desensitization of the PKA-dependent receptor after previous phosphorylation, thus attenuating βAR-mediated response.[15,25,26]

Tse et al[26] showed that cardiac hypertrophy develops in rats treated chronically with ISO stimulation; further, these rats showed decreased magnitude and sensitivity of contractility *in vitro* in response to ISO stimulation. These effects were, related to biochemical alterations, including decreased numbers of βARs, decreased sensitivity and magnitude of adenylate cyclase activity, and decreased cAMP formation. We also clearly showed that PKA activity, but not protein kinase

C (PKC) activity, in the rabbit heart decreased gradually with time after prolonged βAR stimulation.[15] In addition to the study of Tse et al,[26] underlying mechanisms of βAR desensitization to an agonist may be associated with an increased βAR kinase activity.[27] This possibility is supported by the finding that βAR stimulation can significantly increase the expression of βAR kinase 1, whereas βAR blockade decreases the expression.[28]

3. Effect of prolonged βAR stimulation on the vasculature

Despite massive studies on the effects of ISO treatment on the heart, few studies have been performed to evaluate its effects on the vasculature. Pathological cardiac hypertrophy caused by overstimulation of βAR is a potent, independent predictor of cerebrovascular events such as stroke.[29,30]

In diverse vessels, such as the femoral, pulmonary, and carotid arteries, acute stimulation of βAR induces vasodilation.[31] Long-term stimulation of βAR in arteries, however, can induce alterations in vascular contractility.

Previously, we demonstrated that prolonged ISO treatment in rabbits leads to abnormalities in the coronary arterial functions through alterations in the Ca^{2+}-activated K^+ and inward- rectifier K^+ channels in smooth muscle cells. This implies a novel mechanism for vascular dysfunction during cardiac hypertrophy.[14,32] With regard to the rat aorta, Davel et al[33] demonstrated that prolonged ISO stimulation induced endothelial dysfunction and increased vasoconstriction by phenylephrine, an α-adrenergic receptor agonist, due to endothelial dysfunction. They suggested that ISO treatment enhanced the vasoconstrictor response and increased oxidative stress via Endothelial Nitric Oxide Synthase (eNOS) uncoupling, through the β2AR/Giα signaling pathway.[34] Interestingly, we found that βAR stimulation decreased transient Ca^{2+} efflux and attenuated contraction in response to angiotensin II in the rabbit cerebral artery.[35] Possible mechanisms of abnormal response to vasoactivity in different arteries may be due to factors other than biochemical alterations, as shown in the heart. These include the possibility that vascular tissues are vulnerable to oxidative stress, which may disrupt the cytoskeleton further.[35]

4. Differential modulation of the proteome in the heart and cerebral artery during βAR stimulation

To help improve interventions for managing cerebrovascular events during cardiac hypertrophy, here we focus on differences between cardiac and vascular signaling during prolonged βAR stimulation.

Inducible proto-oncogenes encode nuclear transcription factors and activate promoters of many target genes playing a that have roles in cellular functions, adaptive processes, or cell death.[36–38] Prolonged βAR stimulation increases the phosphorylation of Extracellular signal-Regulated Kinase (ERK) increasing expression of *c-fos* and *c-myc* in the cerebral arteries, whereas only *c-fos* expression corresponds to the increased phosphorylation of ERK in the heart. Therefore,

Table 1 – Gene/protein expression profiles in heart and cerebral artery by prolonged βAR stimulation

Identification and functional category		Heart		Cerebral artery		ref.
		increase	decrease	increase	decrease	
Apoptosis/necrosis						
Bcl2l1	Bcl-2-like-protein 1 (Bcl-XL)		–			54
Bcl2l11	Bcl-2-like protein 11	+				54
Bmf	Bcl-2 modifying factor	+				54
Bak1	Bcl-2 antagonist		–			54
Bax	Bcl-2-associated X protein		–			54
Pmaip1	Phorbol-12-myristate-13-acetate-induced protein 1	+				54
Sfn	Stratafin		–			54
Tp53	Tumor protein 53 (p53)		–			54
Apaf1	Apoptotic protease activating factor 1	+				54
Casp1	Caspase-1		–			54
Casp2	Caspase-2 (initiator)		–			54
Casp3	Caspase-3 (effector)	+				54
Casp7	Caspase-7 (effector)		–			54
Casp9	Caspase-9 (initiator)	+				54
Tnfrsf1a	Tumor necrosis factor receptor superfamily 1A		–			54
Tnfsf10	Tumor necrosis factor (ligand) superfamily, 10	+				54
Fas	Tumor necrosis factor receptor superfamily 6	+				54
Stress/energy						
Abcb4	ATP-binding cassette, subfamily B (MDR/TAP) 1A		–			54
Abcc3	ATP-binding cassette protein C3		–			54
Ahr	Aryl-hydrocarbon receptor	+				54
Akt	v-akt murine thymoma viral oncogene homolog 1	+				54
ALDH1A1	Aldehyde dehydrogenase, family 1 member A1			+		35
ALDH2	Aldehyde dehydrogenase, mitochondrial precursor			+		35
ANX6	Annexin VI isoform 1			+		35
ANXA1	Annexin A1 (annexin I)			+		35
ARH	ADP-ribosylhydrolase	+				55
Arnt2	Aryl-hydrocarbon receptor nuclear translocator 2		–			54
ATP5b	ATP synthase subunit β, mitochondrial precursor	+				55
Bcat1	Branched chain amino acid transaminase 1	+				54
CCT2	Chaperonin containing TCP1, subunit 2 (beta)			+		35
DPYSL2	Dihydropytimidinase-like2			+		35
EARH	Ecto ADP-ribosylhydrolase precursor	+				55
EARH	Ecto ADP-ribosylhydrolase precursor	+				55
EF1G	Elongation factor 1-gamma			+		35
GDI2	GDP dissociation inhibitor 2			+		35
GLUD1	Glutamate dehydrogenase			+		35
GSTM5	Glutathione-S-transferase, mu5			+		35
Hif1an	Hypoxia-inducible factor 1-alpha inhibitor		–			54
Hif3a	Hypoxia-inducible factor 3-alpha	+				54
Hsp	Heart shock protein 75 kDa		–			55
Hspa1L	Heat shock 70 kDa, protein 1-like		–			54
Hspb7	Heat shock 27 kDa, cardiovascular	+				54
HspA2	Heat shock 70 kDa, protein 2	+				54
HspA5	Heat shock 70 kDa, protein 5		–			54
HspA8	Heat shock 70 kDa, protein 8 (Hsp73)		–			54
HSPA9	Heat shock protein 9A, mortalin			+		35
IDH1	Isocitrate dehydrogenase 1 (NADP+)			+		35
Lamc	Isoform C of lamin-A/C		–			55
NDUFS1	NADH dehydrogenase (ubiquinone) Fe–S protein 1			+		35
NDUFS8	NADH dehydrogenase (ubiquinone) Fe–S protein 8			+		35
Nos2	Nitric oxide synthase, inducible		–			54
Nr1h4	Nuclear receptor subfamily 1, group H, member 4		–			54
OTUB1	Ubiquitin thioesterase protein OTUB1	+				55
PDIA3	Protein disulfide isomerase family A, member3			+		35
PEA15	Isoform 1 of astrocytic phosphoprotein PEA-15	+				55
Pparγ	Peroxisome proliferator-activated receptor gamma		–			54
Pparα	PPAR alpha		–			54
PPIase	Peptidyl-prolyl cis–trans isomerase E		–			55
RALDH2	Aldehyde dehydrogenase 1A2 isoform 1			+		35
RanGAP	Ran-specific GTPase-activating protein	+				55

Table 1 – (Continued)

Identification and functional category		Heart		Cerebral artery		ref.
		increase	decrease	increase	decrease	
RCN3	Reticulocalbin-3 precursor		−			55
STIP1	Stress-induced phosphoprotein 1			+		35
Ucp3	Mitochondrial uncoupling protein 3		−			54
VEGFA	Vascular endothelial growth factor A	+				54
14-3-3 β/α	Isoform short 14-3-3 protein β/α		−			55
Inflammation						
C3	Complement C3		−			54
C9	Complement C9	+				54
Defb1	Beta-defensin 1	+				54
EHD1	EH-domain containing 1, isoform CRA_a			+		35
Fkbp1	FK506-binding protein 1		−			55
Ifna1	Interferon, alpha 1		−			54
Il-1α	Interleukin-1 alpha	+				54
Il-1β	Interleukin-1 beta	+				54
IL6	Interleukin-6	+				54
MSN	Moesin			+		35
PSME1	Proteasome activator complex subunit 1		−			55
TNFα	Tumor necrosis factor alpha		−			54
TGFb2	Transforming growth factor b2		−			54
Remodeling/fibrosis						
ACTA20	Actin, alpha 2, smooth muscle			+		35
ACTR1A	Actin-related protein 1			+		35
ACTR2	Actin-related protein 2 homolog			+		35
ACTC1	Alpha-actin			+		35
ALB	Albumin			+		35
BMM	Bone marrow macrophage cDNA		−			55
CAPZB	Capping protein (actin filament) muscle Z-line			+		35
Ccl7	Chemokine ligand 7	+				54
COL6A2	Alpha-2-collagen type VI			+		35
CORO1B	Coronin-1B			+		35
Ctgf	Connective tissue growth factor		−			54
Fhl1	Four half Lim domain		−			54
GRIPAP1	GRIP1-associated protein 1			+		35
Il4	Interleukin-4		−			54
Pdlim1	PDZ and LIM domain protein 1	+				55
Reg3b	Regenerating islet-derived 3 beta		−			54
Reg3g	Regenerating islet-derived 3 gamma		−			54
SEPT8	Septin			+		35
Spp1	Osteopontin		−			54
Timp1	Tissue inhibitor metalloproteinase 1		−			54
VIM	Vimentin			+		35
WDR1	WD repeat-containing protein 1 isoform 8			+		35

post-translational modulation appears to progress via different mechanisms in the heart and the cerebral artery.

Although cardiac hypertrophy is not known to be a prerequisite for altered expression of proto-oncogenes *in vivo*,[39] βAR stimulates Gi-dependent PI3 kinase (PI3K) activity and cell growth.[40] In human erythroid progenitors cells, PKCα and PI3Kγ pathways are parallel and converge to activate the *c-fos* and *c-myc* genes.[41]

In addition, decreased signaling of the Ras/Raf/MEK/ERK cascade in the cerebral artery during cardiac hypertrophy can interrupt the actin cytoskeletal network, because Ras/Raf/MEK/ERK is essential for actin-base cytoskeletal organization.[42,43] In contrast, Ras and Raf are activated in the heart during cardiac hypertrophy,[44] and may roles in proliferation and transformation. Decreased PKA activity may possibly contribute indirectly to decreased expressions of the Ras/Raf/MEK/ERK signaling in the cerebral artery, because PKA activity is well known to innately correspond with Ras/Raf activation.[45] However, recent findings also demonstrated that PKA activation does not contribute to Ras/Raf activation.[44,46] Thus we suggested that the underlying mechanism of vascular dysfunction resulting from the decreased expression levels of RhoA and ROCK1 proteins after βAR stimulation.[35] RhoA and ROCK1 are involved in actin-cytoskeletal organization and phosphorylation of myosin light chain producing smooth muscle contraction.[47] The contractility of vascular smooth muscle cells is widely regulated by the cytoskeletal proteome network.[48] Our previous study clearly shows that βAR stimulation disrupts the actin cytoskeletal proteome network through downregulation of RhoA/ROCK1 proteins attenuating angiotensin II-induced contraction in the cerebral artery.[35]

Cardiac or cerebral remodeling by βAR stimulation may involve changes in cellular energy. However, there are a few studies of proteome analysis of βAR stimulated pathways in the heart and the cerebral artery; these studies, revealed

similarities in the main response, including: apoptosis/necrosis, stress/energy, inflammation, and remodeling/fibrosis (also see Table 1). In the heart, a greater number of genes are altered in the category of energy or remodeling, whereas, a greater number of genes involved in cytoskeletal organization are altered in the cerebral artery.

Regarding oxidative stress, expression levels of several cytoprotective chaperones and protein maturation elements are significantly decreased in both the heart and the cerebral artery. Excessive levels of reactive oxygen species (ROS) results in oxidative stress, because the balance between production of ROS and activation of the antioxidant system is essential for controlling homeostasis. Sustained high levels of circulating catecholamines induced by stress can result in cardiotoxicity due to the production of oxygen free radicals.[49] This is supported by several recent findings demonstrating that βAR stimulation increase ROS production in the HEK293 cells, rat cardiac myocytes, and the rat aorta.[50–52] Increased oxidative stress can also lead to DNA damage.[35,53]

Interestingly, in either the heart or the cerebral artery, decreased levels of cytoprotective proteins, including heat shock protein 70/90 and stress-induced-phosphoprotein 1, are more likely due to cause deleterious effects[35,54,55] -rather than increased ROS production. Heat shock proteins are crucial to cellular defense and mitochondrial protection against oxidative stress; these are ubiquitous and highly conserved chaperones are associated with myocardial protection.[56] Oxidative stress activates several kinase signaling pathways, such as PKC, Mitogen-activated protein kinases (MAPK), and PI3K.[57]

In particular, the Bcl-2 like protein 1 and Bak1, which are associated with mitochondria, are significantly altered in the heart.[54] These proteins induce apoptosis by regulating metabolite diffusion across the outer mitochondrial membrane.[58] Apoptosis during cardiac hypertrophy caused by βAR stimulation is of particular interest, as recent literature indicates that deterioration of the hypertrophied heart is linked to progressive loss of cardiomyocytes.[59] Other groups have also shown that inhibition of apoptosis is accompanied by attenuation of heart failure and cardiac hypertrophy, along with increased cardiomyocyte apoptosis prior to the development of significant heart failure.[60,61] Taken together, modulation of apoptosis during cardiac hypertrophy as a preventive for heart failure or stroke may lead to viable therapeutic modalities in the near future.

5.   Conclusion

Epinephrine and norepinephrine injections stimulate αAR and βAR can cause cardiac cell damage to a qualitatively similar extent. In contrast, ISO injection stimulates only βAR can impair the myocardium more severely. Therefore, most of the studies have focused on understanding βAR-mediated signal transduction mechanisms and finding targets to prevent βAR-mediated cardiac remodeling. More recently, βAR overstimulation of vascular structural and function has shown differential effects compared to that of the heart. Therefore, cerebrovascular remodeling and dysfunction reviewed in this study may give a new insight into understanding cerebral

damage after βAR overstimulation, during long-term stress and therapeutic intervention of βAR overstimulation induced cardiovascular events.

Conflicts of interest

There are no conflicts of interest.

Acknowledgments

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