Review article

Adrenergic signaling in heart failure and cardiovascular aging

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\textbf{A B S T R A C T}

Both cardiovascular disease and aging are associated with changes in the sympathetic nervous system. Indeed, mounting evidence indicates that adrenergic receptors are functionally involved in numerous processes underlying both aging and cardiovascular disorders, in particular heart failure. This article will review the pathophysiological role of the sympathetic nervous system in heart failure and cardiovascular aging.

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\textbf{1. Introduction}

Aging is associated with a progressive decline in physiological processes. Cardiovascular disease, and heart failure in particular, remains the leading cause of death among the elderly [1]. Therefore, it is indispensable to explore the molecular mechanisms underlying age-related cardiovascular disorders.

Many of the physiological paradigms developed during the last decades of research in the biological and medical fields may reveal inadequate to understand the aging pathophysiology, in which adaptive and maladaptive mechanisms combine in a new and delicate equilibrium, a thin red line that divides the healthy aging from the fragile individual. The sympathetic nervous system is one of such mechanisms that with aging undergo a series of rearrangements, from baroreflex sensitivity to adrenergic signaling. Over the course of the years the sympathetic nervous system changes its ability to regulate many physiological functions including, not exclusively, the cardiovascular system.

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Cardiac aging is characterized by several complex modifications including diastolic dysfunction, left ventricular hypertrophy, increased risk of atrial fibrillation, valvular degeneration, leading to a decreased maximal exercise capacity. Intriguingly, the sympathetic nervous system plays a key role in both aging and cardiovascular disease. Indeed, adrenergic receptors (ARs) are involved in a variety of pathophysiological processes and mounting evidence indicates their participation in aging and cardiovascular physiopathology (Fig. 1).

2. The sympathetic nervous system

The aging process is accompanied by a series of changes in the autonomic control of the cardiovascular system, favoring heightened cardiac sympathetic tone with parasympathetic withdrawal and blunted cardiovascular baroreflex sensitivity. In a study of older males, the peripheral plasma norepinephrine concentration was approximately 66% higher than that observed in younger men. This finding is explained by the reduction in the rate of clearance of norepinephrine from plasma and 29% higher whole-body norepinephrine spillover to plasma. The cardiac spillover of catecholamines is higher in older than in younger men [2,3]. Different factors can play a role in determining the rate of spillover of norepinephrine from sympathetic nerve terminals to plasma including nerve traffic, pre-junctional modulation, and the rate of catecholamine reuptake, principally via neuronal uptake.

3. Adrenergic receptors and G protein-coupled receptor kinases

Adrenergic receptors (ARs) belong to the guanine nucleotide-binding G protein-coupled receptor (GPCR) superfamily, and are membrane receptors that activate heterotrimeric G proteins. GPCRs consist of one extracellular N-terminal domain, seven membrane-spanning domains, three intra- and three extracellular loops, and one intracellular C-terminal tail. G proteins typically stimulate (Gs protein) or inhibit (Gi protein) the enzyme adenyl cyclase, or activate (Gq protein) phospholipase C (PLC). GPCR signaling is stopped by the family of G protein-coupled receptor kinases (GRKs). GRK-mediated phosphorylation leads to an increased affinity of GPCRs for the arrestin class of proteins, which then uncouples the phosphorylated receptor from G protein and targets the receptor for internalization [4].

Two classes of ARs have been identified: α and β. Phenylephrine is a selective agonist for βAR [5]. The subfamily of α1 ARs (Gq coupled receptors) consists of three highly homologous subtypes, including α1A-, α1B-, and α1D-AR [6]. The α2AR subfamily (coupled to Gi) comprises three subtypes: α2A-, α2B- and α2C-AR [7]. Some species express a fourth α2D-AR [8]. In the βAR family there are three receptor subtypes: β1AR is highly expressed in the heart [9], β2AR is widely distributed throughout the body [10], and β3AR is found, although not exclusively, in the white and brown adipose tissue [11]. All βARs couple primarily to Gi, and subsequent CAMP-related pathways, however under certain conditions they can couple to Goi [12].

Notably, β2AR and β3AR signaling can also occur via mechanisms independent from Gi protein [13]. Additionally, the response to GPCR stimulus can be modified by various parameters, including chronic stimulation, cell hypoxia, acidosis, and aging [14-16].

GRKs have a significant role in the regulation of adrenergic responses. Generally, GRK2 is considered the most important isoform of the 7 mammalian GRKs. Indeed mice with homozygous deletion of GRK2 exhibit embryonic lethality whereas gene ablation for the other GRKs resulted in relatively mild phenotypes [17-19]. GRK-mediated desensitization does not rely only on its catalytic activity but also on protein-protein interactions occurring in different cellular compartments [20]. Both up-regulated and reduced levels of GRK2 may affect cellular function. Alterations in GRK2 activity and expression have been described in numerous diseases, including myocardial infarction [21], Alzheimer’s disease [22], rheumatoid arthritis [23], multiple sclerosis [24], inflammatory pain [25,26], opioid addiction [26], thyroid gland disorders [27], pituitary adenomas [28], ovarian cancer [29], and cystic fibrosis [30].

GRK2 levels in peripheral blood lymphocytes have been reported to mirror changes in kinase expression in other organs under several pathophysiological settings [21]. In particular, GRK2 levels and activity are increased in lymphocytes from hypertensive patients. Impairment of β-adrenergic mediated vasodilation has been reported in both human hypertensive subjects and animal models of hypertension, and such an alteration has been related to the increased GRK2 abundance and activity [31]. Decreased β-adrenergic signaling due to increased GRK2 activity would reduce the vasodilator response, leading to high blood pressure. This view is supported by the inverse correlation of GRK2 expression with blood pressure. Additionally, data from spontaneously hypertensive rats and Dahl salt-sensitive rats confirm increased levels of GRK2 in vascular smooth muscle cells, consistent with the observations in peripheral lymphocytes [32].

As discussed below in the section dedicated to adrenergic signaling and metabolism, elevated GRK2 levels could imply metabolic alterations and lead to insulin-resistance [13,33,34].

To summarize, control of endothelium homeostasis relies on a complex interaction between adrenergic system and nitrosative stress, where specific molecules as GRKs may interplay with and modulate their crosstalk.

4. Adrenergic receptors and cardiovascular aging

The age-associated decrease in catecholamine-responsiveness in the elderly is well established. In particular, an age-related reduction in βAR sensitivity and density has been shown in the myocardium and has been attributed to down-regulation and impaired coupling of βARs to adenyl cyclase [Xiao, 1998 #1544]. The age-linked decline in cardiac βAR response appears to be due to a down-regulation of β1ARs, as reported in experiments in aged explanted human hearts [35]. Additionally, a decreased sensitivity of βARs, measured by isoproterenol-induced changes in the catecholamine-stimulated adenyl cyclase activity, has been shown in the cardiovascular system [10]. βARs...
compartmentalization may also participate in the age-associated decreased βAR responsiveness [36]. Indeed, whereas β1ARs are widely distributed on the plasma membrane, β2ARs are usually located in the transverse tubules, containing specialized proteins that couple membrane depolarization (excitation) to calcium-mediated myofilament shortening (contraction) [10,37–39]. Henceforward, the localization of β2AR in cardiac cells leads to the generation of spatially restricted cAMP production, affecting calcium-dependent proteins that control the contraction of myofilaments [40,41]. Several conditions presenting a decreased myocardial function can elicit activity from the sympathetic nervous system (SNS) that eventually diverts blood flow to critical organs and increases cardiac output. The main players involved in such system are catecholamines, whose release is strictly orchestrated by the GPCR system, relating the adrenal gland and the heart [42].

Most of the modifications in the sympathetic nervous system occurring with aging, including decreased βAR responsiveness, increased circulating catecholamines, and overall hyposensitivity to adrenergic stress, are also observed in patients with failing hearts. Moreover, young people are more reactive to isoprotanol-induced increase in blood flow than elderly subjects [31].

Vascular tone is finely regulated by the intimal (endothelial cells, EC) and the medial (vascular smooth muscle cells, VSMC) layers [43–45] and both EC and VSMC express β2AR [46–48]. The age-related decline in β2AR function and successive cAMP generation is observed in diverse cardiovascular disorders, including atherosclerosis, hypertension, vascular insufficiency, and orthostatic hypotension, all conditions with significant mortality and morbidity [49–53]. The increased incidence of restenosis and atherosclerosis in aged people may also rely on the age-associated deterioration in βAR-mediated CAMP production, since cAMP may inhibit VSMC proliferation [45,54]. A reduced responsiveness with age has been also reported for αAR in healthy subjects [55], with potential implications for reduced muscle blood flow and augmented blood pressure during exercise [56–58].

During the late 90’s the idea that the EC possessed functional βARs became more and more evident, and soon this system was seen as a therapeutic target [59,60]. Nevertheless, the proangiogenic effects of the β2AR was firstly identified by our group in 2005 [47]. The picture was completed, when we finally demonstrated that the endothelium is the source of catecholamines that stimulate in a paracrine manner endothelial βARs [46]. These findings were further corroborated by the observation that also endothelial progenitor cells express β2AR and that the ability of this receptor to signaling to eNOS promotes angiogenesis through multiple mechanisms [61]. This ability is lost during aging. Strategies that aim to restore β2AR signaling in models where it is lost are effective to restore also impaired angiogenesis [47,62]. For instance, in aging the enhancement of βAR signaling leads to the correction of impaired angiogenesis [10,63,64].

The overall incidence of cardiac disorders including heart failure, left ventricular hypotrophy, and arrhythmias, increases considerably with age [1,65]. Elderly people appear to be particularly predisposed to the development of heart failure: such a diagnosis is the leading cause of hospitalizations in people >65 years of age [1,15]. Additionally, alterations in ventricular relaxation and filling have been described with aging [64,66].

The prevalence of atrial fibrillation reaches values of 17.8% in people >85 years [1,67,68]. The development of a non-regular pattern of electrical activity might have detrimental consequences in hearts that are relatively stiff and relax slowly [1,69,70].

Likewise, the prevalence of left ventricular hypertrophy increases with rising blood pressure and body mass index [71,72] and studies in normotensive people indicate that myocardial wall thickness increases progressively with age [73].

In the vasculature an age-linked increase in intimal thickening has been reported, accompanied by luminal dilatation and reduction in distensibility, resulting in increased vessel stiffness. Indeed, pulse wave velocity, a noninvasive index of vascular stiffening, increases with age and has been associated with structural alterations in the media [13,44,49,64,74–76].

5. Adrenergic signaling in heart failure

The SNS has prominent effects on cardiac physiology, including increases in atrioventricular conduction (positive dromotropy), heart rate (positive chronotropy), cardiac contractility (positive inotropy), and cardiac relaxation (positive lusitropy). Crucially, the SNS plays a crucial role in the regulation of vascular tone by controlling both peripheral resistance and cardiac output [77,78].

Heart failure is a chronic syndrome in which the heart is unable of pumping an adequate supply of blood to meet the metabolic requirements of the body or generating the required elevated ventricular filling pressures to maintain output [34]. Despite considerable advances in the treatment of heart failure, such a disease still represents a severe social and clinical burden [42,79,80]. The cardiovascular system is best viewed as a complex dynamic system, continually adapting to optimize organ perfusion. During heart failure, diverse neurohormonal mechanisms are triggered to maintain cardiac output [4]. Heart failure is considered a progressive disease that begins long before signs or symptoms become clinically evident: initially there is a complex adaptive neurohormonal activation – required to compensate for cardiac dysfunction – which includes nervous system, renin-angiotensin-aldosterone system, endothelin, natriuretic peptides, and vasopressin. The process progressively becomes maladaptive, leading to increased mechanical stress on the failing heart and causing harmful electrical and structural events [81–83]. Thus, β-blockers, Angiotensin II AT1 receptor blockers, angiotensin-converting enzyme inhibitors, and mineralocorticoid receptor antagonists represent cornerstones for the treatment of patients with heart failure. Given the decreased myocardial contractility observed in heart failure and the positive inotropic response obtained with βAR agonists, it was initially attractive to attempt to overcome the βAR desensitization in order to ameliorate cardiac function via the stimulation of the adrenergic system [84]. Several authors also provided experimental evidence that β2AR overexpression might have been a new treatment for heart failure [85,86]. Other investigators demonstrated that the overexpression of β1AR [87] or β2AR [88] leads to cardiomyopathy and heart failure, as reflected by decreased cardiac function, increased fibrosis, cardiomyocyte apoptosis, and overall mortality.

The central part of the adrenal gland, called adrenal medulla, is the main source of catecholamines and is comprised of groups of adrenergic and noradrenergic chromaffin cells and, to a lesser extent, ganglionic neurons [42,89]. The adrenal gland can be seen as a specialized sympathetic ganglion, receiving inputs from the sympathetic nervous system via pre-ganglionic fibers. Yet, the adrenal gland directly secretes neurohormones into the bloodstream [89].

The sympathetic overdrive observed in heart failure correlates with a higher risk of arrhythmias and left ventricular dysfunction [90]. Moreover, augmented levels of catecholamines can cause myocardial damage via enhanced cardiac oxygen demand and by increasing peroxidative metabolism [91] and ultimately leading to structural alterations, including focal necrosis and inflammation, increased collagen deposition, and interstitial fibrosis [92–94].

Systemically circulating or locally released catecholamines trigger two main classes of ARs: α1AR and β2AR, causing vasocostriction and vasodilatation, respectively [46,47,95]. With aging, such a fine equilibrium is progressively shifted toward increased...
vasoconstriction, most likely due to a defective vasodilatation in response to βAR stimulation.

Increased basal levels of circulating catecholamines have been observed both in heart failure and with advancing age, mirrored by a decrease in the number of high-affinity βARs. These findings suggest that these alterations might be attributable to βAR desensitization rather than an actual reduction in βAR density [10,21,96].

Both expression and activity of GRK2 increase in vascular tissue with aging [97]. Equally important, an impairment in βAR-mediated vasorelaxation has been observed in hypertensive patients [31] and in animal models of hypertension [75,97]; such an alteration has been related to increased GRK2 abundance and activity. Transgenic overexpression of GRK2 in the vasculature impairs βAR signaling and the vasodilator response, eliciting a hypertensive phenotype in rodents. This aspect has been confirmed in humans: GRK2 expression correlates with blood pressure and impaired βAR-mediated adenylcyclase activity [97]. Additionally, variants of the gene encoding for β2AR have been associated with longevity [10].

The decreased βAR-mediated response has been attributed to different mechanisms, including an attenuation of PKA activation, an impaired generation of cyclic AMP, a reduced receptor density, and a less efficient coupling to adenylcyclase [10]. However, currently there is no single molecular or cellular factor that can fully explain the decline in βAR function. Nonetheless, the etiology seems to be most likely associated with alterations in the ability of βAR to respond to agonists at the cellular level.

6. Adrenergic signaling and metabolism

The adrenergic system is involved in regulating several metabolic pathways. Increased circulating catecholamines and activation of the different ARs present in the various organs produce important metabolic responses, including increased gluconeogenesis by the liver to provide substrate for the brain, increased lipolysis and elevated levels of fatty acids in plasma, and modulation of insulin secretion by pancreatic islets of Langerhans. Such responses are detrimental to the functioning of different organs (e.g., the heart). Metabolic modifications including insulin resistance, altered glucose and lipid metabolism, and mitochondrial dysfunction represent common features of many conditions involving adrenergic overdrive. Notably, these alterations are seen in a number of different pathological conditions and are generally highly correlated with the level of activation of the sympathetic system.

Chronic βAR stimulation induces insulin resistance and in this context the β2AR has a key role in overall glucose homeostasis by modulating pancreatic islet hormone secretion as well as liver and muscle glucose homeostasis. Short- and long-term stimulation of the β2AR has been associated with the modulation of fatty acid and glucose metabolism [98]. Indeed, acute treatment with β2AR agonists of myocytes or skeletal muscle increases glucose uptake to levels comparable to those seen after insulin stimulation [99].

A potential mechanism for β2AR function in insulin resistance involves the activation of PI3K and its downstream signal pathway and in particular the phosphorylation and inactivation of TBC1D4 by AKT [100]. TBC1D4 inhibits the translocation to the plasma membrane of the glucose transporter type 4 (GLUT4) [100]. Moreover, TBC1D4 is also targeted by AMPK, which represents a pivotal mechanism in the regulation of insulin-independent glucose uptake [100,101]. Strikingly, higher levels of AMPK phosphorylation and activity are seen in response to βAR stimulation [102,103] as a result of changes in the AMP/ATP ratio or activation of upstream AMPK kinases [104]. Besides, in vivo studies show a greater efficiency of carvedilol, a non-selective βAR antagonist, in ameliorating myocardial insulin sensitivity and glucose extraction in an animal model of heart failure, compared to the selective β1AR antagonist metoprolol [105]. Chronic adrenergic stimulation, as seen during heart failure, would be detrimental by mechanisms involving mechanisms such as JNK, β-arrestins and GRKs [4,106].

Insulin resistance highly correlates with adrenergic function [75,107–111]. In both type 2 diabetes mellitus (T2DM) and heart failure circulating insulin levels are elevated, causing a persistant stimulation of insulin receptors [52,112–117]. Hyperactive insulin signaling can accelerate adverse left ventricular remodeling [72,118]. Recently, insulin has been demonstrated to directly impair adrenergic pathways for contractile function via an insulin receptor/β2AR signaling complex [114].

In numerous conditions associated with insulin resistance, such as hypertension and T2DM, there are elevated GRK2 levels [97,119]. In vitro experiments demonstrated that insulin increases GRK2 levels, causing GRK2–IRS1 association [97,120]. On these bases, GRK2 inhibition has been proposed to be beneficial. Indeed, chronic treatment of spontaneously hypertensive rats with an inhibitor of GRK2 kinase activity ameliorates glucose homeostasis and decreases blood pressure [120].

Growing evidence indicates that GRKs can exert different effects within the cell depending on the stimulus, cell type, and localization [97,121]. In this sense, we were the first to demonstrate a mitochondrial localization for GRK2 [122], later confirmed by other investigators [123], establishing a functional role for GRK2 in organelle biogenesis and ATP production [122,124].

7. Sympathetic system and remodeling

Histopathologic evaluation in murine hearts reveals subendocardial and interstitial fibrosis, vacuolization of cytoplasm and mineralization [16,125–127]. Furthermore, morphometric analysis of cardiomyocytes demonstrates a progressive hypertrophy, increased apoptosis alongside with fibrosis and amyloid deposition [128].

Several organs undergo fibrotic remodeling as a function of age resulting in overall decreased functionality. The precise mechanisms leading to the age-dependent accumulation of collagen have yet to be fully identified. Mounting evidence reveals the emerging role of collagen cross-linkers and matrix metalloproteinases (MMP) in the turnover of collagens, finely regulated by proteolytic MMP activity and their endogenous tissue inhibitors (TIMPs) [129,130]. All of these mechanisms are modulated by neuroendocrine activation and βAR signal transduction modifications. Indeed, chronic sympathetic activation significantly contributes to progression from compensated left ventricular hypertrophy to myocardial dysfunction through detrimental cardiac matrix remodeling. Besides, sympathetic overactivity achieved via β1AR overexpression (β1TG mouse) [87,131] is accompanied by interstitial matrix remodeling and turnover by inducing MMP/TIMPs. In β1TG pro-collagen type-I and type-III mRNA and interstitial collagen protein expression are progressively increased from four-fold to 17-fold at 12 months compared to 3 months of age. In β1TG mice at 5 months of age with compensated cardiac hypertrophy, an increased deposition of type-I and –II collagen fibers surrounding the cardiomyocytes is observed [131]. In contrast, the β1TG group at 12 months of age exhibits an asymmetrical distribution of the collagen fibers with disruption of the collagen network structure [132,133].

A positive correlation between MMP-2 and noradrenaline in patients with heart failure has been demonstrated, also corroborated by in vitro experiments in human cardiac fibroblasts [134], supporting thereby the link between sympathetic stimulation and collagen turnover.
8. Conclusion

With aging the sympathetic nervous system undergoes a series of rearrangements, from baroreflex sensitivity to adrenergic signaling, and over the course of the years, it changes its ability to regulate countess physiological functions. The exaggerated sympathetic activity observed in aging, discussed in this report, has to be put into the perspective of a whole scenario, in which the effectors of catecholamines are also undergoing a plasticity with the ultimate intent of allowing an adequate signaling.

Contributors

Both authors contributed significantly to the work, read the manuscript, and agreed to its submission.

Conflict of interest

None declared.

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References

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