



Review

Acute neurohumoral modulation of diastolic function

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ARTICLE INFO

Article history:

Received 9 September 2008

Received in revised form 26 October 2008

Accepted 27 October 2008

Available online 5 November 2008

Keywords:

Diastole

Cardiovascular physiology

Heart failure

Angiotensin-II

Endothelin-1

ABSTRACT

Diastole plays a central role in cardiovascular homeostasis. Its two main determinants, myocardial relaxation and passive properties of the ventricular wall, are nowadays regarded as physiological mechanisms susceptible of active modulation. Furthermore, diastolic dysfunction and heart failure with normal ejection fraction (previously called diastolic heart failure) are two subjects of major clinical relevance and an intense area of research. The role of several neurohumoral mediators like angiotensin-II and endothelin-1 on the modulation of diastolic function was systematically described as having only chronic deleterious effects such as cardiac hypertrophy and fibrosis. However, over the last years a growing body of evidence described a new role for several peptides on the acute modulation of diastolic function. In the acute setting, some of these mediators may have the potential to induce an adaptive cardiac response. In this review, we describe the role of angiotensin-II, endothelin-1, nitric oxide, urotensin-II and ghrelin on the acute modulation of diastolic function, emphasizing its pathophysiological relevance. Only a thorough understanding of diastolic physiology as well as its active modulation, both in the acute and chronic settings, will improve our knowledge on diastolic dysfunction and allow us to solve the enigmas of heart failure with normal ejection fraction.

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1. Introduction

Diastolic function has a central role in cardiovascular homeostasis. Its importance is obvious not only as a physiological function of the normal cardiac cycle (allowing the ventricle to relax and fill with blood) but also as a dysfunctional component on

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important cardiovascular diseases such as heart failure with normal ejection fraction (HFNEF) [31].

The two main determinants of diastolic function are myocardial relaxation and passive properties of the ventricular wall (see [16,24,31] for comprehensive reviews on diastole and diastolic dysfunction). The former is modulated by load, inactivation (including mechanisms related to calcium homeostasis and myofilaments properties) and non-uniformity whereas the latter depends on cardiomyocytes themselves (mainly the cytoskeleton) and cardiac extracellular matrix.

The modulation of diastolic function by neurohumoral agents has been an area of intense investigation over the last decade. The adrenergic system is the best described neurohumoral mechanism capable of acutely modulate both systolic and diastolic functions. Furthermore, peptides such as endothelin (ET) and angiotensin-II (AngII) were systematically regarded as having only chronic deleterious effects on systolic and diastolic function, contributing to ventricular remodeling with myocardial hypertrophy and fibrosis. Notwithstanding these ominous effects, over the recent years there was an increasing body of evidence linking several neurohumoral mediators to the acute modulation of diastole, with special emphasis on the active myocardial relaxation and passive properties of the cardiac muscle [33,34,50].

In this way, this work aims to provide an up-to-date description on the latest neurohumoral mechanisms found to acutely modulate diastolic function. First, the most important intracellular mechanisms playing a role on diastole will be briefly described. Thereafter, the acute effects of some neurohumoral mediators on diastolic function will be reviewed. A better understanding of diastolic physiology and its modulation will probably contribute to unmask some of the current enigmas of HFNEF and to offer a broader perspective on potential and clinically relevant approaches for the treatment of diastolic dysfunction and HFNEF.

2. Intracellular mechanisms modulating diastolic function

2.1. Diastolic calcium homeostasis

Calcium homeostasis plays a determinant role in myocardial relaxation [31]. Indeed, calcium extrusion from the cytosol is crucial for a normal diastole and the mechanisms contributing to reduce intracellular calcium levels are altered in diastolic dysfunction. In the human heart, the main processes responsible for calcium extrusion are its uptake by the sarcoplasmic reticulum calcium-ATPase (SERCA) pump and the activity of the sarcolemmal sodium-calcium exchanger (NCX). SERCA pump activity is inhibited by phospholamban (PLB) in its dephosphorylated form. However, PLB phosphorylation removes its inhibitory effect on SERCA and promotes a faster rate of relaxation due to increased SERCA activity. In this way, increased activity of SERCA by direct activation or phosphorylation of PLB by protein kinases allows the ventricle to accelerate myocardial relaxation. However, decreased SERCA activity or hypophosphorylated PLB contribute to a slow rate of tension decline.

2.2. Sarcomeric proteins

Key regulatory myofilament proteins such as troponins and myosin binding protein C (MyBP-C) also play a role modulating cardiac diastolic properties. Phosphorylation of troponin (Tn)-I by PKA [26], PKC [45] and PKG [30] is responsible for enhanced relaxation resulting from the decreased myofilament calcium-sensitivity. Interestingly, a recent work [10] demonstrated that myofilament desensitization induced by PKA requires the presence of another sarcomeric protein, MyBP-C. The latter seems to be an

important mediator of diastole [51] but further research is needed to allow a better understanding of a dynamic interaction between these myofilament proteins on the modulation of diastolic function.

Titin is a giant sarcomeric protein increasingly recognized as an important determinant of myocardial stiffness and contributor to the pathophysiology of heart failure [18]. Indeed, passive stiffness can be adjusted by modulating the mechanical properties of titin's extensible region mainly by phosphorylation and changes in intracellular calcium levels.

PKA-dependent phosphorylation of titin's N2B unique sequence reduces passive tension of both animal [64] and human [28] cardiomyocytes. This represents one of the mechanisms contributing to the improvement of cardiac diastolic properties upon β adrenergic stimulation. A recent work [60] suggests that PKC may also phosphorylate titin but at present is unclear whether only PKA can phosphorylate titin or whether PKC is also effective.

Calcium and the protein S100A1, a calcium-binding protein, also contribute to the modulation of myocardial passive stiffness. Together, they inhibit titin-actin interaction, one of the determinants of passive myocyte stiffness, in a calcium-dependent manner [63]. However, future work is required to clarify the array of rapid adjustment mechanisms involving titin as well as to understand their physiological role in the modulation of diastolic function.

3. Acute neurohumoral mediators of diastolic function

3.1. Endothelin

Endothelin-1 was isolated in 1988 and was characterized as the most potent endogenous vasoconstrictor ever identified [65]. At first regarded as "the bad guy of circulatory control" [61], ET-1 is nowadays considered an important regulator of cardiovascular homeostasis (see [6] for a comprehensive review on this topic).

ET-1 has predominantly autocrine and paracrine actions by binding to two main subtypes of G-protein coupled receptors, ET_A and ET_B. ET_A receptor activation evokes vasoconstriction [41], mitogenesis [46] and increased inotropism [22,25] whereas ET_B receptor stimulation is associated with vasodilatation and growth inhibitory effects [40] associated with apoptosis [47]. The latter was further subdivided in ET_{B1} and ET_{B2} receptor subtypes: the ET_{B1} receptor is predominantly expressed in the vascular and endocardial endothelium and its stimulation elicits vasodilatation [12] and a negative inotropic effect [32] and the ET_{B2} receptor is located on vascular smooth muscle and myocardial cells, mediating vasoconstriction [59] and positive inotropism [32]. The ET_B receptor is also responsible for the pulmonary clearance [15] and endothelial reuptake [48] of circulating ET-1. It is important to remember that ET-1 plasma levels in healthy subjects are very low, in the femtomolar range, being unlikely that this peptide acts as a circulating hormone. However, there is a tonic paracrine release of ET-1 from endocardial endothelial cells inasmuch as ET receptor blockade elicited a decrease in systolic contractility demonstrated by a reduction in the rate of left ventricular pressure development [39,55]. Despite the vast knowledge about the role of ET-1 on cardiovascular physiology, its diastolic effects have only recently started to be uncovered. Accurate evidence was gathered in the setting of isolated muscle preparations and hemodynamic studies, inasmuch as in the intact heart it is difficult to differentiate between effects of ET-1 on intrinsic myocardial diastolic properties from those related to load changes [16,35] and coronary vasoconstriction [13,23]. Indeed, in the intact heart, effects of ET-1 on diastolic function might result from direct actions of the peptide and from indirect effects that are mainly the consequence of coronary and/or systemic vasoconstriction. Such a complex

regulation of myocardial diastole with potentially simultaneous opposing effects of ET-1 might explain why no conclusive effects on the modulation of diastolic function by ET-1 in the intact heart have been reported.

3.1.1. The effects of ET-1 on myocardial relaxation

In isolated muscle preparations, ET-1 elicits a dose-dependent (0.1, 1 and 10 nM) acceleration of the rate of tension decline (increased dT/dt_{min}) [33,38]. To understand the physiologic mechanisms of this positive lusitropic effect, studies were performed in isolated rabbit papillary muscles. We could observe that the increased lusitropism in the presence of ET-1 (1 nM) was inhibited after nonselective ET_A/ET_B receptor blockade with PD-145065 and even reversed when a selective ET_A receptor antagonist (BQ-123) was used. Furthermore, selective ET_B receptor inhibition with BQ-788 in the presence of ET-1 showed a tendency to enhance the positive lusitropic response [33]. Interestingly, the acceleration of the rate of tension decline elicited by ET-1 does not depend on the integrity of the endocardial endothelium [3,4] nor on the presence of NO or prostaglandins [4].

To further clarify the role of ET_B receptor on the ET-1-induced positive lusitropism, another experimental protocol was conducted. We demonstrated that selective ET_{B1} receptor stimulation slowed myocardial relaxation (decreased dT/dt_{min}) through the release of NO and prostaglandins, whereas there was a faster relaxation upon preferential ET_{B2} receptor stimulation [32]. More recently, we demonstrated that the lusitropic effect of selective ET_B receptor stimulation is dependent in the integrity of the endocardial endothelium, being negative when it is intact and positive when it is damaged [32].

Overall, these results suggest that the ET-1-induced positive lusitropic response is predominantly mediated by ET_A receptors and that the interaction with ET_{B1} and ET_{B2} stimulation and the endothelium may also contribute to the modulation of this response.

3.1.2. The effects of ET-1 on passive myocardial properties

It is well established that chronically elevated levels of ET-1 are associated with myocardial hypertrophy and fibrosis, leading to an increase in myocardial stiffness and thus compromising the diastolic function of the heart [5,11]. However, the acute diastolic effects of ET-1 are being progressively unraveled with obvious implications for the understanding of diastolic physiology.

Experiments performed by our group in isolated rabbit papillary muscles and human auricular strips revealed that ET-1 dose dependently (using the concentrations 0.1, 1 and 10 nM) increases myocardial distensibility (decreases myocardial stiffness) in acutely loaded cardiac muscles by binding to ET_A receptors and activating the Na^+/H^+ exchanger (NHE). This effect was not inhibited by selective ET_B receptor inhibition with BQ-788, which is considered to predominantly block the ET_{B2} receptor subtype [33]. In the clinical setting, upon ET_A receptor stimulation and in the presence of cardiac overload, the ventricle can reach higher filling volumes at lower filling pressures, with a more efficient recruitment of the preload reserve. This may represent a quite powerful adaptation mechanism of cardiac diastolic function in situations of acute cardiac overload, decreasing the odds of backwards congestion.

Subsequent studies demonstrated that the ET-1-induced decrease in myocardial stiffness also requires an intact endocardial endothelium [3,4] and active endothelial ET_{B1} receptors (although selective ET_{B1} or ET_{B2} direct stimulation did not elicit any effect on this parameter) [3]. This suggests that acute modulation of passive diastolic properties of the myocardium by ET-1 is probably dependent on a complex balance and interaction between ET_A

and ET_{B1} receptors stimulation and requires a functional endocardial endothelium to determine the overall physiologic response.

In a recent work, we demonstrated that after blocking NO and prostaglandins synthesis with N^G -nitro-L-arginine (L-NNA) and indomethacin, respectively, the ET-1-induced decrease in resting tension (increase in myocardial distensibility) was no longer observed [4]. Since NO and prostaglandins are important endothelial mediators and are known to be released by the endothelium upon selective ET_{B1} receptor stimulation [6], these results suggest that these substances probably play an important role modulating the acute diastolic effects of ET-1 in a complex cross-talk between the endothelium and cardiac muscle.

3.2. Angiotensin-II

The octapeptide AngII plays a determinant role in the maintenance of cardiovascular homeostasis, as well as in different cardiovascular diseases such as hypertension and heart failure (see [43] for a thorough review of AngII physiological and pathological effects in the cardiovascular system).

The existence and function of a local renin–angiotensin system (RAS) in the heart is now well established [49]. Furthermore, the predominant physiological role of the cardiac RAS is probably the integration of several stimuli mediating adaptive responses to myocardial stress (such as the response to myocyte stretch) as well as to modulate the local effects of growth and proliferative stimuli. Therefore, the local RAS might as well contribute to the modulation of myocardial diastolic function in the acute setting.

AngII stimulates two types of G protein-coupled receptors: AT_1 receptor, associated not only with acute vasoconstriction and positive inotropism but also with chronic ventricular remodeling, by means of myocardial hypertrophy and fibrosis; and the AT_2 receptor, commonly associated with anti-proliferative and proapoptotic effects and vasodilatation [1].

Notwithstanding the deleterious effects of chronically elevated levels of AngII over both systolic and diastolic functions, the acute diastolic response to AngII was systematically overlooked.

3.2.1. The effects of AngII on myocardial relaxation

The early findings on AngII-dependent modulation of myocardial relaxation evidenced a prolonged relaxation time in the cat ventricle as well as in other species [21,42,44,54]. However, our recent results in isolated rabbit papillary muscles and in situ rabbit hearts suggest that AngII (using increasing concentrations from 10^{-7} to 10^{-5} M) exerts a positive lusitropic effect characterized by an increase in the peak rate of tension decline (dT/dt_{min}) [8,34]. We also demonstrated that the AngII-induced acceleration of myocardial relaxation was completely abolished with losartan (selective competitive AT_1 receptor antagonist) and significantly attenuated after nonselective endothelin ET_A/ET_B receptor and selective ET_A receptor blockade as well as after selective removal of the endocardial endothelium. These results suggest that the acceleration of the rate of tension decline induced by AngII is indeed dependent on AT_1 receptor activation and probably results from an acute interaction between the local renin–angiotensin system and the endothelin system with the endothelium playing an important role in its modulation.

In a subsequent study [9], our group demonstrated that the selective stimulation of rabbit myocardial AT_2 receptors was associated with a slowing of myocardial relaxation and thus a negative lusitropic effect that was completely abolished after removal of the endocardial endothelium and significantly attenuated with hydroxocobalamin (a NO scavenger) and Hoe-140 (an antagonist of bradykinin B_2 receptors). A link between AT_2 receptor signaling pathway and stimulation of endothelial bradykinin B_2

receptors (with activation of constitutive nitric oxide synthase) was already described in the heart [29]. Overall, these findings demonstrated that selective AT₂ receptor-stimulation is associated with a negative lusitropic effect modulated by the endocardial endothelium and mediated by bradykinin B₂ receptors through NO release.

3.2.2. The effects of AngII on passive myocardial properties

In a recent work, our group described for the first time a significant AngII concentration dependent (10^{-7} – 10^{-5} M) decrease of myocardial stiffness [34]. Indeed, AngII acutely decreased passive tension by 46% in the isolated muscle and LV diastolic pressures by 40–44% in the intact heart. To further clarify this finding, selective AT₁ (losartan and ZD-7155) and AT₂ (PD-123,319) receptors antagonists were used. Interestingly, only AT₁ receptor blockade inhibited the increase in myocardial distensibility. We also demonstrated that this effect was completely blunted upon PKC inhibition and significantly attenuated after NHE inhibition. These results suggest that the previously described acute decrease of myocardial stiffness by AngII requires AT₁ receptor activation and is mediated by PKC and NHE. It is tempting to hypothesize that a possible mechanism accounting for this effect may be a PKC-dependent phosphorylation of titin promoting a decrease in myocardial stiffness in a way similar to titin's phosphorylation by PKA. However, future research is required to further clarify this issue.

3.3. Nitric oxide

Research work performed over the last decade evidenced that NO plays an important role in cardiovascular homeostasis, as well as in the pathophysiology of heart diseases such as heart failure (see [53] for a comprehensive review). However, NO-induced diastolic effects were only recently unmasked and remain at present an interesting matter of investigation.

The original observations on the effects of NO in the setting of isolated papillary muscles were described as a relaxation-hastening effect, characterized by reduced peak isometric tension because of earlier onset of relaxation. These results were also evident in experimental preparations using a whole animal heart model, where both exogenous and endogenous NO were found to induce a faster rate of myocardial relaxation [19,20]. Likewise, the same effects were observed in the normal human heart upon intracoronary injection of sodium nitroprusside during cardiac catheterization [50].

A novel and interesting diastolic effect of NO was demonstrated in 1994 in isolated cardiomyocytes [57] and in the intact human left ventricle, in which [50] NO was shown to promote a shifting of the length-tension and LV diastolic pressure–volume relations to the right, respectively. Physiologically, these results clearly indicate that NO signaling favors a more distensible cardiomyocyte and a more compliant left ventricular chamber. On the other hand, in the setting of isolated animal heart the presence of N^G-monomethyl-L-arginine (a specific inhibitor of nitric oxide synthase) results in elevation of LV end-diastolic pressure suggesting a stiffer ventricle [52]. Due to the raised myocardial concentration of cGMP elicited by NO, one of the mechanisms suggested to mediate the NO-dependent increase in diastolic distensibility is the phosphorylation of Tn-I by PKG eliciting a reduction of myofilamentary calcium sensitivity [56]. As recently described, a PKG-mediated phosphorylation of titin is also likely to account for the observed effect [27].

Taken together, these results suggest that NO is probably an important regulator of diastolic function in a complex acute interplay among coronary perfusion, coronary endothelium and

diastolic LV performance through relaxation-hastening and distensibility-increasing effects. Acutely, elevation of cardiac workload and shear stress promotes endothelial NO release which optimizes LV diastolic function by prolonging the diastolic time interval and allowing the ventricle to accommodate higher volumes at lower filling pressures.

3.4. Urotensin-II

Urotensin-II is a cyclic peptide isolated in 1985 which was recently introduced as a mediator in cardiovascular homeostasis and disease (see [66] for a comprehensive review on the role of U-II on cardiovascular and renal physiology). However, after more than 20 years of research, the precise role of U-II on diastolic function still deserves future investigation.

To further clarify the diastolic effects of U-II and some of their underlying mechanisms, the study model of isolated rabbit papillary muscles was employed [14]. Regarding myocardial relaxation, we found a concentration-dependent (10^{-8} – 10^{-6} M) negative lusitropic effect that was not affected by damaging the endocardial endothelium nor blocking NO and prostaglandins synthesis. However, the most interesting finding of this study was the evidence of a dose-dependent acute increase in myocardial distensibility upon U-II addition. Blocking U-II receptors with urantide and NO synthesis with L-NNA totally inhibited this effect whereas cyclooxygenase inhibition with indomethacin significantly attenuated it. Interestingly, endocardial endothelium removal did not alter the distensibility-increasing effect of U-II. Taken together, these results suggest that the U-II-induced increase in myocardial distensibility depends on U-II receptor stimulation and NO and prostaglandins release probably from the microvascular coronary endothelium, which is a recognized alternative source of these mediators [7]. These findings demonstrated the likelihood of U-II as a mediator of an acute adaptation mechanism that allows the ventricle to reach higher diastolic volumes at lower filling pressures. Nevertheless, one should not neglect that chronically elevated levels of U-II contribute to cardiac fibrosis and hypertrophy [2,66] and accelerate the atherosclerotic process [62] leading to cardiovascular function impairment.

3.5. Ghrelin

Ghrelin is a 28-amino acid peptide mostly synthesized in the stomach with important metabolic and homeostatic roles by providing an endocrine link between stomach, hypothalamus and pituitary (see [37] for a thorough review on the physiological, pathological and therapeutic roles of ghrelin). Ghrelin also participates in several aspects of physiologic and pathologic states of the cardiovascular system [36]. However, its role in diastole still remains poorly understood. In a recent work [58], our group described the negative lusitropic effect of ghrelin (using concentrations of 10^{-9} – 10^{-6} M) in isolated rat papillary muscles, consisting on a slower rate of tension decline and an earlier onset of myocardial relaxation. The decreased lusitropism was not affected by endocardial endothelium damage or blocking NO and prostaglandins synthesis. Nevertheless, the premature onset of relaxation induced by ghrelin was blunted by inhibition of prostaglandins release and exacerbated by NO synthase inhibition, but not affected by a dysfunctional endocardial endothelium. These results suggest that the acute modulation of the onset of myocardial relaxation by ghrelin may depend on a complex interaction between NO, prostaglandins and the endothelium that should be addressed in future research work. Interestingly, antagonizing the growth hormone secreta-

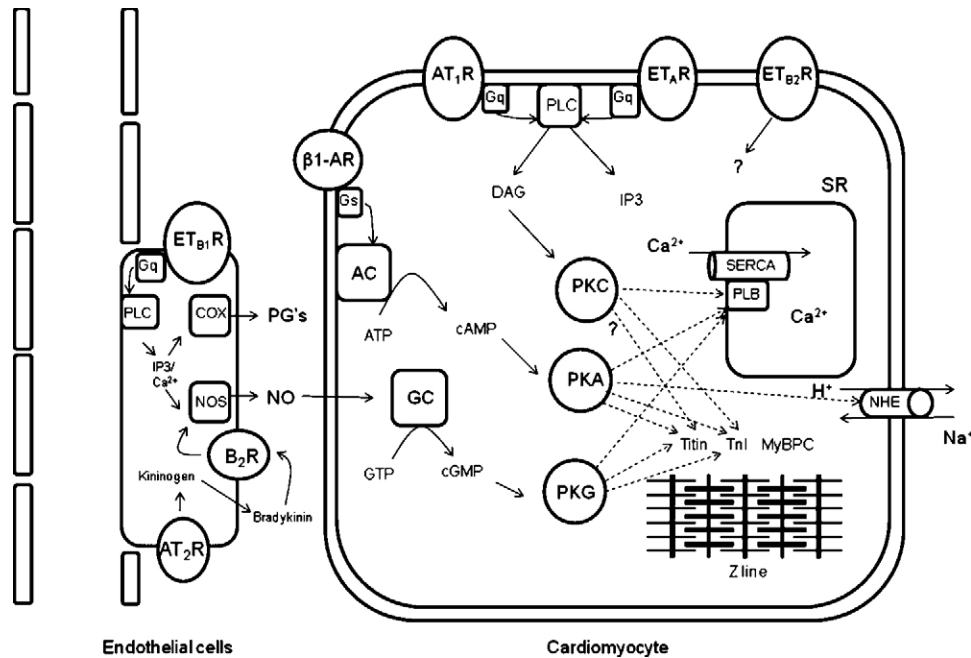


Fig. 1. Major signaling pathways described to contribute to the acute modulation of diastolic function. Dashed arrows represent phosphorylations. AC, adenylyl cyclase; AR, adrenergic receptor; AT₁R, AT₁ receptor; AT₂R, AT₂ receptor; B₂R, bradykinin B₂ receptor; cAMP, cyclic AMP; cGMP, cyclic GMP; COX, cyclooxygenase; DAG, diacylglycerol; ET_AR, ET_A receptor; ET_{B1}R, ET_{B1} receptor; ET_{B2}R, ET_{B2} receptor; GC, guanylyl cyclase; Gq, Gs, G-protein subunits; IP₃, inositol 1,4,5-triphosphate; MyBPC, myosin binding protein C; NHE, Na⁺/H⁺ exchanger; NO, nitric oxide; NOS, NO synthase; PG's, prostaglandins; PKA, PKC, PKG, protein kinases A, C and G, respectively; PLB, phospholamban; PLC, phospholipase-C; SERCA, SR Ca²⁺ pump; SR, sarcoplasmic reticulum; TnI, troponin I.

Table 1

Overview of the acute effects of several neurohumoral mediators on diastolic function.

Mediator	Effect	Reference(s)
Endothelin-1	ET _A receptor stimulation Positive lusitropism independent of endocardial endothelium, NO and prostaglandins Decrease in myocardial stiffness dependent on active ET _{B1} receptors, intact endocardial endothelium, NO and prostaglandins and Na ⁺ /H ⁺ exchanger activation	[3,4] [3,4,33]
	ET _{B1} receptor stimulation Negative lusitropism dependent on NO and prostaglandins release and intact endocardial endothelium	[32]
	ET _{B2} receptor stimulation Positive lusitropism (unknown mechanism)	[32]
Angiotensin-II	AT ₁ receptor stimulation Positive lusitropism dependent on ET _A and ET _B receptor integrity and intact endocardial endothelium Decrease in myocardial stiffness dependent on Na ⁺ /H ⁺ exchanger and protein kinase C	[8,34] [34]
	AT ₂ receptor stimulation Negative lusitropism dependent on intact endocardial endothelium, NO and bradykinin B ₂ receptor	[9]
Nitric oxide	Earlier onset of relaxation ("relaxation-hastening effect") Positive lusitropism Decrease in myocardial stiffness	[19,20] [19,20] [50,57]
Urotensin-II	Negative lusitropism independent of intact endocardial endothelium, NO and prostaglandins Decrease in myocardial stiffness dependent on urotensin-II receptor stimulation, NO and prostaglandins	[14] [14]
Ghrelin	Earlier onset of relaxation blunted by inhibition of prostaglandins release and exacerbated by NO synthase inhibition Negative lusitropism independent of intact endocardial endothelium, NO and prostaglandins	[58] [58]

gogue-receptor (GHS-R1a), one of the most important mediators of ghrelin action, did not modify the negative lusitropic and relaxation-hastening effect of ghrelin, implying that some of its actions are mediated by other receptors distinct from the GHS-R1a. The GHS-R1b is also expressed in the myocardium [17] and thus is a putative candidate for mediating some of the cardiac actions of ghrelin.

4. Conclusion

The concept that diastole is not a merely passive phenomenon but is instead under active control is now a common ground in both clinical and basic research settings. Indeed, the recognition that diastole itself can be actively modulated has been steering current research so that its pathophysiological mediators are

unveiled and new pharmacological approaches are designed and implemented in the setting of diastolic dysfunction and HFNEF. As for the adrenergic system, many other neurohumoral mechanisms commonly associated with the progression of heart failure syndrome have been described to acutely and positively modify cardiac function. In regard to myocardial diastolic properties, AngII and ET-1 figure as two unquestionable new paradigms of such modulation (schematically depicted in Fig. 1). Being mostly regarded as positive inotropic and vasoconstrictor mechanisms in acute heart failure but decompensatory ones in the chronic heart failure setting, their ability to acutely modify diastolic function has been widely dismissed. This gap has been filled by our recent observations that AngII and ET-1 can significantly improve diastolic function by means of increasing relaxation velocity and decreasing myocardial stiffness in a time-frame that is too short to alter the ECM. In the clinical ground, these effects may translate into an enhanced capacity for the ventricle to increase its end-diastolic volume at concurrently lower filling pressures. In the acute heart failure setting and upon neurohumoral activation, ventricular wall stress decreases and the odds of congestion are significantly attenuated. The same effects are ascribed to NO and, more recently, to U-II (see Table 1 for an overview). Though, the net effect in the human myocardium integrating the actions of all these peptides should always be regarded as a complex and still incompletely understood issue that also depends on the integrity of the endothelium, probably leading to different diastolic cardiac behaviors in the healthy state and in the diseased heart. An observation that further underscores the importance of diastolic function modulation in cardiovascular homeostasis is the redundancy of effects among neurohumoral mediators that activate different intracellular signaling pathways. Multiple signaling mediators seem to converge on a similar effect in the regulation of myofilamentary calcium sensitivity and myocardial distensibility. Either through direct or indirect stimulation of PKA (adrenalin), PKC (AngII and ET-1) and/or PKG (NO and U-II), these mediators share the acutely-decreasing effect of myocardial stiffness, highlighting the existence of several ways to achieve a common end: an enhanced diastolic function.

References

- [1] Batenburg WW, Garrelts IM, Bernasconi CC, Juillerat-Jeanneret L, van Kats JP, Saxena PR, et al. Angiotensin II type 2 receptor-mediated vasodilation in human coronary microarteries. *Circulation* 2004;109:2296–301.
- [2] Bousette N, Pottinger J, Ramli W, Ohlstein EH, Dhanak D, Douglas SA, et al. Urotensin-II receptor blockade with SB-611812 attenuates cardiac remodeling in experimental ischemic heart disease. *Peptides* 2006;27:2919–26.
- [3] Bras-Silva C, Leite-Moreira AF. Obligatory role of the endocardial endothelium in the increase of myocardial distensibility induced by endothelin-1. *Exp Biol Med* (Maywood) 2006;231:876–81.
- [4] Bras-Silva C, Monteiro-Sousa D, Duarte AJ, Guerra M, Fontes-Sousa AP, Moura C, et al. Nitric oxide and prostaglandins—important players in endothelin-1 induced myocardial distensibility. *Physiol Res* 2008;57:165–74.
- [5] Brown RD, Ambler SK, Mitchell MD, Long CS. The cardiac fibroblast: therapeutic target in myocardial remodeling and failure. *Annu Rev Pharmacol Toxicol* 2005;45:657–87.
- [6] Brunner F, Bras-Silva C, Cerdeira AS, Leite-Moreira AF. Cardiovascular endothelins: essential regulators of cardiovascular homeostasis. *Pharmacol Ther* 2006;111:508–31.
- [7] Brutsaert DL. Cardiac endothelial-myocardial signaling: its role in cardiac growth, contractile performance, and rhythmicity. *Physiol Rev* 2003;83:59–115.
- [8] Castro-Chaves P, Roncon-Albuquerque Jr R, Leite-Moreira AF. Endothelin ETA receptors and endothelium partially mediate the positive inotropic and lusitropic effects of angiotensin II. *Eur J Pharmacol* 2006;544:91–6.
- [9] Castro-Chaves P, Soares S, Fontes-Carvalho R, Leite-Moreira AF. Negative inotropic effect of selective AT(2) receptor stimulation and its modulation by the endocardial endothelium. *Eur J Pharmacol* 2008;578:261–9.
- [10] Cazorla O, Szilagyi S, Vignier N, Salazar G, Krämer E, Vassort G, et al. Length and protein kinase A modulations of myocytes in cardiac myosin binding protein C-deficient mice. *Cardiovasc Res* 2006;69:370–80.
- [11] Clozel M, Salloukh H. Role of endothelin in fibrosis and anti-fibrotic potential of bosentan. *Ann Med* 2005;37:2–12.
- [12] de Nucci G, Thomas R, D'Orleans-Juste P, Antunes E, Walder C, Warner TD, et al. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelin-derived relaxing factor. *Proc Natl Acad Sci USA* 1988;85:9797–800.
- [13] Ezra D, Goldstein RE, Czaja JF, Feuerstein GZ. Lethal ischemia due to intracoronary endothelin in pigs. *Am J Physiol* 1989;257:H339–43.
- [14] Fontes-Sousa AP, Bras-Silva C, Pires AL, Monteiro-Sousa D, Leite-Moreira AF. Urotensin II acutely increases myocardial length and distensibility: potential implications for diastolic function and ventricular remodeling. *Naunyn Schmiedeberg Arch Pharmacol* 2007;376:107–15.
- [15] Fukuroda T, Fujikawa T, Ozaki S, Ishikawa K, Yano M, Nishikibe M. Clearance of circulating endothelin-1 by ETB receptors in rats. *Biochem Biophys Res Commun* 1994;199:1461–5.
- [16] Gillebert TC, Leite-Moreira AF, De Hert SG. Load dependent diastolic dysfunction in heart failure. *Heart Fail Rev* 2000;5:345–55.
- [17] Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, et al. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J Clin Endocrinol Metab* 2002;87:2988.
- [18] Granzier H, Wu Y, Siegfried L, LeWinter M. Titin: physiological function and role in cardiomyopathy and failure. *Heart Fail Rev* 2005;10:211–23.
- [19] Grocott-Mason R, Anning P, Evans H, Lewis MJ, Shah AM. Modulation of left ventricular relaxation in isolated ejecting heart by endogenous nitric oxide. *Am J Physiol* 1994;267:H1804–13.
- [20] Grocott-Mason R, Fort S, Lewis MJ, Shah AM. Myocardial relaxant effect of exogenous nitric oxide in isolated ejecting hearts. *Am J Physiol* 1994;266:H1699–705.
- [21] Ishihata A, Endoh M. Pharmacological characteristics of the positive inotropic effect of angiotensin II in the rabbit ventricular myocardium. *Br J Pharmacol* 1993;108:999–1005.
- [22] Ishikawa T, Li LM, Shinmi O, Kimura S, Yanagisawa M, Goto K, et al. Characteristics of binding of endothelin-1 and endothelin-3 to rat hearts. Developmental changes in mechanical responses and receptor subtypes. *Circ Res* 1991;69:918–26.
- [23] Karwatowska-Prokopcuk E, Wennmalm A. Effects of endothelin on coronary flow, mechanical performance, oxygen uptake, and formation of purines and on outflow of prostacyclin in the isolated rabbit heart. *Circ Res* 1990;66:46–54.
- [24] Kass DA, Bronzwaer JG, Paulus WJ. What mechanisms underlie diastolic dysfunction in heart failure? *Circ Res* 2004;94:1533–42.
- [25] Kelso EJ, McDermott BJ, Silke B, Spiers JP. Endothelin(A) receptor subtype mediates endothelin-induced contractility in left ventricular cardiomyocytes isolated from rabbit myocardium. *J Pharmacol Exp Ther* 2000;294:1047–52.
- [26] Kentish JC, McCloskey DT, Layland J, Palmer S, Leiden JM, Martin AF, et al. Phosphorylation of troponin I by protein kinase A accelerates relaxation and crossbridge cycle kinetics in mouse ventricular muscle. *Circ Res* 2001;88:1059–65.
- [27] Krüger M, dos Remedios C, Linke WA. Titin phosphorylation by protein kinases A and G in normal and failing human hearts decreases myocardial passive stiffness. *Circulation* 2007;116:IL301.
- [28] Krüger M, Linke WA. Protein kinase-A phosphorylates titin in human heart muscle and reduces myofibrillar passive tension. *J Muscle Res Cell Motil* 2006;27:435–44.
- [29] Kurisu S, Ozono R, Oshima T, Kambe M, Ishida T, Sugino H, et al. Cardiac angiotensin II type 2 receptor activates the kinin/NO system and inhibits fibrosis. *Hypertension* 2003;41:99–107.
- [30] Layland J, Li JM, Shah AM. Role of cyclic GMP-dependent protein kinase in the contractile response to exogenous nitric oxide in rat cardiac myocytes. *J Physiol* 2002;540:457–67.
- [31] Leite-Moreira AF. Current perspectives in diastolic dysfunction and diastolic heart failure. *Heart* 2006;92:712–8.
- [32] Leite-Moreira AF, Bras-Silva C. Inotropic effects of ETB receptor stimulation and their modulation by endocardial endothelium, NO, and prostaglandins. *Am J Physiol Heart Circ Physiol* 2004;287:H1194–9.
- [33] Leite-Moreira AF, Bras-Silva C, Pedrosa CA, Rocha-Sousa AA. ET-1 increases distensibility of acutely loaded myocardium: a novel ETA and Na⁺/H⁺ exchanger-mediated effect. *Am J Physiol Heart Circ Physiol* 2003;284:H1332–9.
- [34] Leite-Moreira AF, Castro-Chaves P, Pimentel-Nunes P, Lima-Carneiro A, Guerra MS, Soares JB, et al. Angiotensin II acutely decreases myocardial stiffness: a novel AT1, PKC and Na⁺/H⁺ exchanger-mediated effect. *Br J Pharmacol* 2006;147:690–7.
- [35] Leite-Moreira AF, Gillebert TC. Nonuniform course of left ventricular pressure fall and its regulation by load and contractile state. *Circulation* 1994;90:2481–91.
- [36] Leite-Moreira AF, Rocha-Sousa A, Henriques-Coelho T. Cardiac, skeletal, and smooth muscle regulation by ghrelin. *Vitam Horm* 2008;77:207–38.
- [37] Leite-Moreira AF, Soares JB. Physiological, pathological and potential therapeutic roles of ghrelin. *Drug Discov Today* 2007;12:276–88.
- [38] Li K, Stewart DJ, Rouleau JL. Myocardial contractile actions of endothelin-1 in rat and rabbit papillary muscles. Role of endocardial endothelium. *Circ Res* 1991;69:301–12.
- [39] MacCarthy PA, Grocott-Mason R, Prendergast BD, Shah AM. Contrasting inotropic effects of endogenous endothelin in the normal and failing human heart—studies with an intracoronary ETA receptor antagonist. *Circulation* 2000;101:142–7.

- [40] Mallat A, Fouassier L, Preaux AM, Gal CS, Raufaste D, Rosenbaum J, et al. Growth inhibitory properties of endothelin-1 in human hepatic myofibroblastic Ito cells. An endothelin B receptor-mediated pathway. *J Clin Invest* 1995;96:42–9.
- [41] Marsault R, Feolde E, Frelin C. Receptor externalization determines sustained contractile responses to endothelin-1 in the rat aorta. *Am J Physiol* 1993;264:C687–93.
- [42] Mattiazzi A. Positive inotropic effect of angiotensin II. Increases in intracellular Ca^{2+} or changes in myofilament Ca^{2+} responsiveness? *J Pharmacol Toxicol Methods* 1997;37:205–14.
- [43] Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol* 2007;292:C82–97.
- [44] Moravec CS, Schluchter MD, Paranandi L, Czerska B, Stewart RW, Rosenkranz E, et al. Inotropic effects of angiotensin II on human cardiac muscle in vitro. *Circulation* 1990;82:1973–84.
- [45] Noland TAJ, Guo X, Raynor RL, Jideama NM, Averyhart-Fullard V, Solaro RJ, et al. Cardiac troponin I mutants. Phosphorylation by protein kinases C and A and regulation of Ca^{2+} -stimulated MgATPase of reconstituted actomyosin S-1. *J Biol Chem* 1995;270:25445–54.
- [46] Ohlstein EH, Arleth A, Bryan H, Elliott JD, Sung CP. The selective endothelin ETA receptor antagonist BQ123 antagonizes endothelin-1-mediated mitogenesis. *Eur J Pharmacol* 1992;225:347–50.
- [47] Okazawa M, Shiraki T, Ninomiya H, Kobayashi S, Masaki T. Endothelin-induced apoptosis of A375 human melanoma cells. *J Biol Chem* 1998;273:12584–92.
- [48] Ozaki S, Ohwaki K, Ihara M, Fukuroda T, Ishikawa K, Yano M. ETB-mediated regulation of extracellular levels of endothelin-1 in cultured human endothelial cells. *Biochem Biophys Res Commun* 1995;209:483–9.
- [49] Paul M, Mehr AP, Kreutz R. Physiology of local renin–angiotensin systems. *Physiol Rev* 2006;86:747–803.
- [50] Paulus WJ, Vantrimpont PJ, Shah AM. Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans. Assessment by bicoronary sodium nitroprusside infusion. *Circulation* 1994;89:2070–8.
- [51] Pohlmann L, Kröger I, Vignier N, Schlossarek S, Krämer E, Coirault C, et al. Cardiac myosin-binding protein C is required for complete relaxation in intact myocytes. *Circ Res* 2007;101:928–38.
- [52] Prendergast BD, Sagach VF, Shah AM. Basal release of nitric oxide augments the Frank–Starling response in the isolated heart. *Circulation* 1997;96:1320–9.
- [53] Rastaldo R, Pagliaro P, Cappello S, Penna C, Mancardi D, Westerhof N, et al. Nitric oxide and cardiac function. *Life Sci* 2007;81:779–93.
- [54] Salas MA, Vila-Petroff MG, Palomeque J, Aiello EA, Mattiazzi A. Positive inotropic and negative lusitropic effect of angiotensin II: intracellular mechanisms and second messengers. *J Mol Cell Cardiol* 2001;33:1957–71.
- [55] Shah AM. Paracrine modulation of heart cell function by endothelial cells. *Cardiovasc Res* 1996;31:847–67.
- [56] Shah AM, McCarthy PA. Paracrine and autocrine effects of nitric oxide on myocardial function. *Pharmacol Ther* 2000;86:49–86.
- [57] Shah AM, Spurgeon HA, Sollott SJ, Talo A, Lakatta EG. 8-Bromo-cGMP reduces the myofilament response to Ca^{2+} in intact cardiac myocytes. *Circ Res* 1994;74:970–8.
- [58] Soares JB, Rocha-Sousa A, Castro-Chaves P, Henriques-Coelho T, Leite-Moreira AF. Inotropic and lusitropic effects of ghrelin and their modulation by the endocardial endothelium, NO, prostaglandins, GHS-R1a and KCa channels. *Peptides* 2006;27:1616–23.
- [59] Sudjarwo SA, Hori M, Tanaka T, Matsuda Y, Okada T, Karaki H. Subtypes of endothelin ETA and ETB receptors mediating venous smooth muscle contraction. *Biochem Biophys Res Commun* 1994;200:627–33.
- [60] van der Velden J, Narolska NA, Lamberts RR, Boontje NM, Borbély A, Zaremba R, et al. Functional effects of protein kinase C-mediated myofilament phosphorylation in human myocardium. *Cardiovasc Res* 2006;69:876–87.
- [61] Vanhoutte PM. Endothelin-1. A matter of life and breath. *Nature* 1994;368:693–4.
- [62] Watanabe T, Kanome T, Miyazaki A, Katagiri T. Human urotensin II as a link between hypertension and coronary artery disease. *Hypertens Res* 2006;29:375–87.
- [63] Yamasaki R, Berri M, Wu Y, Trombitás K, McNabb M, Kellermayer MS, et al. Titin–actin interaction in mouse myocardium: passive tension modulation and its regulation by calcium/S100A1. *Biophys J* 2001;81:2297–313.
- [64] Yamasaki R, Wu Y, McNabb M, Greaser M, Labeit S, Granzier H. Protein kinase A phosphorylates titin's cardiac-specific N2B domain and reduces passive tension in rat cardiac myocytes. *Circ Res* 2002;90:1181–8.
- [65] Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;332:411–5.
- [66] Zhu YC, Zhu YZ, Moore PK. The role of urotensin II in cardiovascular and renal physiology and diseases. *Br J Pharmacol* 2006;148:884–901.