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Endothelium-driven myocardial growth or nitric oxide at the crossroads

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Abstract

Endothelium lining the coronary vasculature and the heart chambers is a dynamic sensor that serves a variety of functions including bi-directional communications with cardiac myocytes. Among endothelium-released factors nitric oxide exerts multifactorial effects on various cell types in the heart and may play a role in growth of the vasculature and myocardial hypertrophy. This review summarizes new data regarding the endothelium-to-myocyte signaling focusing on its role in regulation of cardiac hypertrophy through a nitric oxide mediated paracrine signal.

Introduction

In the early seventeenth century William Harvey pictured the heart as a pump that forces blood to move throughout the body. Under normal physiological conditions the heart delivers adequate blood supply sufficient to meet metabolic needs of the body, while increases in oxygen demand, as occur under stress or exercise, are met by increasing the heart rate, stroke volume and contractility. Persistent hemodynamic stress leads to cardiac enlargement typically accompanied by increased muscle thicknesses, a growth event referred to as cardiac hypertrophy. The *sine qua non* of cardiac hypertrophy is the increase in cardiac myocyte size, rather than the increase in cell number (Dorn et al. 2003). Yet, precise mechanisms responsible for the induction of cardiac myocyte hypertrophy and factors initiating and maintaining this process have not been defined. Recent studies have suggested that myocyte hypertrophy has to be accompanied by an increase in the heart vasculature in order to avoid the development of heart failure secondary to insufficient oxygen delivery to the increased muscle mass. Tantalizingly, the increase in heart vasculature may not only support the increase in myocyte mass but may actually induce this process. In this review, we will discuss the growth-modulating properties of the endothelium-to-myocytes signaling emphasizing the role of nitric oxide (NO).

Cardiac Endothelium

Cardiac endothelium contains two types of endothelial cells: the vascular endothelial cells lining coronary blood vessels, and the endocardial endothelial cells, lining the cardiac chambers. Although there are similarities in structure and functions between these two

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endothelium types, there are also significant differences, including embryological origins, cytoskeleton organization, receptor-mediated function, cell junctions (Kuruvilla and Kartha 2003). A close spatial relationship between endothelial cells and cardiomyocytes - no cardiac myocyte is more than 2-3 μm from an endothelial cell- facilitates signal transmission between the two cell types (Shah and MacCarthy 2000).

Endothelium contributes to cardiovascular homeostasis by regulating vascular permeability, vascular resistance and myocardial stiffness. Endothelium releases locally vasoconstricting and vasodilating factors such as NO, endothelin and prostaglandins, pro- and anticoagulant factors, and various growth factors including fibroblast growth factor (FGFs), vascular endothelial growth factor (VEGFs) and platelet-derived growth factor BB (PDGF-BB), thereby modulating vascular resistance, tissue water content and myocardial blood content. Furthermore, the luminal surface of endothelial cells harbors a number of enzymes, including angiotensin converting enzyme (ACE), capable of affecting numerous parameters of myocardial and vascular function.

Yet despite this plethora of biologically active molecules, little is known about the effect of endothelial signaling on myocytes. Two possible signaling mechanisms from the cardiac endothelium to cardiomyocytes have been proposed: (1) a paracrine pathway, described as “stimulus-secretion-contraction coupling”, and (2) a transendothelial ion transport, described as “ionic blood-heart barrier” (Fransen et al. 2001, 2003). The cardiac inotropic effect induced by endothelial paracrine factors is associated with a reduction in a myofilament responsiveness to Ca^{2+} rather than an alteration in Ca^{2+} transients (Shah et al. 1996). A consequence of this rather unusual mode of action is a disproportionate effect on myocardial relaxation and diastolic performance. However, apart from their influence in regulating myocardial contractile behavior, endothelial cell-derived factors may also affect myocardial growth and hypertrophy. Among factors released by the endothelium, NO acts as an autocrine factor promoting endothelial cell sprouting and vessel growth as well as a paracrine factor affecting cardiac myocytes.

Nitric oxide in the heart

Initially identified in the 1970s as a potent vasodilator, NO has been recognized in the last 20 years as an important regulator of myocardial function, either indirectly by dilating the systemic vasculature or directly by modulating contractile performance, metabolism, cell growth and survival (Massion et al. 2003, 2005). The precise nature of these effects has been controversial, especially owing to the extensive use of non-isoform-selective inhibitors of NO synthases (NOS) and various NO-donor compounds (Seddon et al. 2007).

In the heart, there are at least two important cellular sources of NO: coronary and endocardial endothelial cells and cardiac myocytes. Endothelial NOS (eNOS) is expressed predominantly in the cardiac endothelium with only minimal expression in cardiac myocytes. eNOS expression is quite heterogeneous, both with respect to the level of expression, i.e., greater in coronary arteries and endocardium compared with capillaries and coronary vein endothelium, and its subcellular localization (Andries et al. 1998). In the endothelium, eNOS-caveolin-1 interaction in caveolae is critical for eNOS activity. Caveolin not only serves as a scaffold to ensure coupling of eNOS to specific receptors in caveolae, but also maintains the enzyme in an inactive state through direct inhibitory allosteric interaction (reviewed in Feron and Balligand 2006, Gratton et al. 2004). The physiological stimuli for NO release from coronary vascular endothelial cells may be flow-induced shear stress and cyclical mechanical deformation of the endothelium that occurs during the cardiac cycle (Shah and MacCarthy 2000). At a much lower level, eNOS is expressed in cardiac myocytes in plasmalemmal and T-tubular caveolae, where is associated with caveolin-3 (Massion et al. 2003). Neuronal NOS

(nNOS) is expressed in the sarcoplasmic reticulum of cardiomyocytes (Xu et al. 1999). The distinct subcellular expression of eNOS and nNOS in cardiomyocytes suggests discrete coupling mechanisms, limited to a local cytosolic environment able to activate different pathways following enzyme activation (Feron and Balligand 2006, Williams et al. 2006).

NO affects myocytes in a number of different ways (Figure 1). Most of the effects of NO involve activation of cytoplasmic soluble guanylyl cyclases (sGSs) that increases cytoplasmic cGMP content (Moncada et al. 1991). However, several cGMP-independent actions of NO have also been identified, and these may be particularly relevant when large amounts of NO are produced by inducible NOS (iNOS) (Stamler et al. 1992). The main target proteins for cGMP in myocardium are cGMP-dependent protein kinases (PKGs) and cGMP-stimulated and -inhibited cyclic nucleotide phosphodiesterases (cGsPDE and cGiPDE, respectively). The former pathway potentially alters myocyte function through phosphorylation of various kinases, whereas the latter modulates intracellular levels of cAMP. In the heart several effects have been attributed to cGMP, including modulation of sarcolemmal Ca^{2+} influx, changes in the action potential characteristics, reduction in myofilament Ca^{2+} -sensitivity, reduction in O_2 consumption (reviewed in Shah and MacCarthy 2000).

eNOS-derived nitric oxide and its role in angiogenesis

Endothelial cells treated with VEGF release NO in a biphasic process- acutely (minutes to hours) and chronically (>24 h). The chronic NO production is sustained by the increase in eNOS (mRNA and protein) levels induced by VEGF in a dose-dependent manner (Hood et al. 1998). In animal models, NO released after VEGF administration leads to reduction in systemic blood pressure and increase in vascular permeability (Hariawala et al. 1996; Horowitz et al. 1997). Similarly, intracoronary VEGF administration in patients with coronary artery disease also decreases arterial blood pressure and systemic vascular resistance (Hendel et al. 2000, Henry et al. 2001). However, repeated administrations of VEGF lead to a rapid tachyphylaxis with no appreciable changes in systemic hemodynamics (Lopez et al. 1997).

eNOS activity is essential for physiologic neovascularization that occurs in response to tissue ischemia. This was well documented in eNOS^{-/-} mice, where angiogenesis was severely impaired in the setting of hindlimb ischemia (Mees et al. 2007, Yu et al. 2005). Interestingly, in the same animal model arteriogenesis or collateral growth does not seem to require NO, although NO is necessary for collateral vessel vasodilatation (Mees et al. 2007). eNOS transgenic overexpression enhanced angiogenesis and blood flow recovery in an ischemic model (Amano et al. 2003). Cardiovascular agents modulating endogenous NO bioactivity, such as ACE inhibitors, statins, and phosphodiesterase inhibitor, may be useful in promoting angiogenesis in ischemic tissue (Simons 2000). Statins (HMG-CoA reductase inhibitors) stimulate eNOS activation via the protein kinase Akt pathway (Kureishi et al. 2000).

Nitric oxide and cardiomyocyte contractility

Stimulation of eNOS activity by administration of substance P in humans alters LV systolic pressure and increases LV end-diastolic distensability, suggesting a reduction in LV stiffness (Paulus et al. 1995). Similar effects have also been observed in isolated cardiomyocyte in culture treated with cGMP-analogues, PKG or NO donors (Shah et al. 1994). In general, it is thought that low levels of NO have a positive inotropic effect, while high levels have a negative inotropic effect. However, defining the “low” and “high” NO concentrations is a difficult task requiring consideration of NO source (i.e. exogenous, endogenous or paracrine from endothelial cells), experimental setting (*in vivo* vs. *in vitro*) and intracellular interactions with ROS or potential scavengers (i.e. myoglobin) (reviewed in Massion et al. 2003). For example, the NO donor SNAP at lower concentration (1 μM) stimulates contractility of isolated cardiomyocytes, whereas a higher concentration (100 μM) depresses it. The positive inotropic

effect is mediated by cAMP, whereas negative effect is induced by cGMP activation (Vila-Petroff et al. 1999). The lusitropic effect of NO on cardiac myocytes, accelerating relaxation and reducing diastolic tone, has been attributed to a reduction in myofilament Ca^{2+} responsiveness caused by PKG-induced phosphorylation of troponin I (TnI) (Layland et al. 2002; Shah et al. 1994).

If eNOS is solely responsible for diastolic performance, then its disruption would induce severe alterations of cardiac function. However, despite general hypertension developed in eNOS deficient mice, no baseline cardiac contractility differences in eNOS^{-/-} vs. wild type mice have been reported (Gyurko et al. 2000, Martin et al. 2006). Compensatory expression of atrial natriuretic peptide (ANP) in eNOS^{-/-} might be responsible for the lusitropic effect (Gyurko et al. 2000). Reciprocally, mice overexpressing eNOS exhibit hypotension, increased hypertensive response to NOS inhibitors and resistance to NO/cGMP-mediated vasodilators (van Deel et al. 2007, Yamashita et al. 2000). At the same time, the increase in NO production in mice overexpressing eNOS attenuates congestive heart failure after myocardial infarction due to reduction of cardiac afterload via increased diastolic reserve (Jones et al. 2003).

Anti-hypertrophic effect of nitric oxide

The anti-hypertrophic properties of NO were first demonstrated in cultured cardiac myocytes, where NO donors were shown to inhibit the hypertrophic response to growth factors. The anti-hypertrophic NO effect is a result of a negative modulation of nuclear factor-activated T cell (NFAT), a pro-hypertrophic pathway (Fiedler et al. 2002) that includes the ubiquitously expressed Ca^{2+} /calmodulin-activated serine/threonine phosphatase calcineurin. Sustained elevations of intracellular Ca^{2+} concentration activate calcineurin which in turn dephosphorylates cytoplasmic latent transcription factor NFAT, leading to its shift to the nucleus and induction of the hypertrophic program of gene expression. In cardiomyocytes, NO elevates cGMP and PKG activity, which reduces cellular Ca^{2+} availability and calcineurin activity by inhibiting L-type Ca^{2+} channels, thus suppressing NFAT activation (Figure 1, left side). Calcineurin-NFAT pathway can also be inhibited by NO/cGMP-induced suppression of calcineurin A subunit expression (Fiedler and Wollert 2004). In addition, NO donor SNAP has been shown to downregulate the expression of the cytoskeleton-associated muscle LIM protein (MLP) in ET-1 stimulated neonatal rat cardiomyocytes thereby restraining hypertrophic stimulation (Heineke et al. 2003). Interestingly, even in the absence of exogenous NO, constitutively generated NO negatively modulates ET-1-induced cardiac hypertrophy by a concurrent activation of NOS through the stimulation of ET-receptor subtype ET_B (Cheng et al. 2005). Finally, NO inhibits ET-1-induced *c-fos* expression by preventing ERK and AP-1 activation, and β -MHC expression.

Several studies support the role of NO in preventing pathological cardiac remodeling. In failing human myocardium the reduced NO production and lower NOS expression suggest that an increase in myocardial NOS levels might be beneficial in rescuing the maladaptive hypertrophy (Paulus 2001). In line with this hypothesis, eNOS gene delivery to the heart attenuates cardiac hypertrophy post myocardial infarction (Smith et al. 2005). The cardioprotective role of NO is further associated with reduced myocardial fibrosis and apoptosis, and suppression of oxidative stress-mediated signaling pathways. Furthermore, a novel therapeutic avenue might consider a new generation of NO donors able to restore eNOS signaling pathway in preventing maladaptive cardiac hypertrophy (Ruiz-Hurtado et al. 2007).

Interestingly, eNOS deficient mice do not develop myocardial hypertrophy until after 2 months of age (Barouch et al. 2002). Since eNOS^{-/-} mice are hypertensive one could argue that the hypertrophy simply accrues as a response to hypertension. However, eNOS^{-/-} mice subjected to a myocardial infarction developed more hypertrophy in the remote (normal) myocardium

than wild type mice, even when the blood pressure was normalized with hydralazine (Scherrer-Crosbie et al. 2001). Similarly, eNOS^{-/-} mice subjected to a transverse aortic constriction showed greater LV hypertrophy than wild-type counterparts (Ichinose et al. 2004). However, using a similar pressure overload model Takimoto et al. observed less hypertrophy in eNOS^{-/-} mice and greater LV dilation in wild type as result of eNOS uncoupling and increased synthesis of reactive oxygen species (Takimoto et al. 2005). A possible explanation for this discrepancy might be related to the degree (severe vs. modest) of pressure overload in this model.

On the other hand, mice overexpressing eNOS in endothelium under preproendothelin-1 promoter, developed less myocardial hypertrophy after treatment with isoproterenol (Ozaki et al. 2002). However, transgenic eNOS overexpression under eNOS promoter improved survival and decreased pulmonary edema after myocardial infarction, but did not extend cardiac hypertrophy or improve LV function (Jones et al. 2003). This may have been due to decreased afterload rather than a direct effect on the myocardium.

Pro-hypertrophic effect of nitric oxide

The increase in NO production as a result of increased endothelial cell mass may promote myocardial hypertrophy in the absence of the usual “hypertrophic” stimuli such as increased afterload (Tirziu et al. 2007). In this study, stimulation of angiogenesis in the normal mouse heart not subjected to any hemodynamic load resulted, with a 3 week delay, in increase in cardiomyocyte size and overall increase in the heart size and mass. No increase in myocyte proliferation was detected. The increase in the heart size was significantly but not fully prevented by concurrent treatment with an eNOS inhibitor L-NAME demonstrating that NO is responsible for about a half of the observed effect (Tirziu et al. 2007). The mechanism of this NO-induced myocardial hypertrophy is not clear. One potential explanation might be related to the NO role in the N-end rule pathway, an ubiquitin-dependent proteolytic degradation of intracellular proteins in which destabilization of N-terminal residues functions as an essential determinant of degradation signal.

In cardiomyocytes, Gq/Gi protein complexes mediate hypertrophy signal. Regulators of G-protein signaling (RGS proteins) act as GTPase activating proteins toward α -subunit of heterotrimeric G proteins. By deactivating G α , RGS proteins negatively modulate Gq/Gi-mediated cardiovascular signaling in hypertrophy and contractility (reviewed in Wieland and Mittmann 2003). The canonical effector molecules of activated GTP-bound family G α subunits are β -isoforms of phospholipase C (PLC- β). Downstream to PLC- β the G α -mediated signal is propagated by several pathways including inositol trisphosphate (IP₃), calcium, diacylglycerol (DAG), PKC (reviewed in Hubbard and Hepler 2006). Among RGS proteins, RGS4 up-regulation is related to maladaptive hypertrophy in human failing hearts (Mittmann et al. 2002) and its transgenic expression in cardiac myocytes prevented cardiac hypertrophy in response to pressure overload (Rogers et al. 1999). Additionally, RGS4 overexpression stops hypertrophic stimulation induced by phenylephrine and endothelin-1 (Tamirisa et al. 1999) in cultured cardiomyocytes. Therefore, RGS4 appears to be an important regulator of cardiac hypertrophy mainly based on G α q mediated signal (Rogers et al. 2001).

RGS4 cytoplasmic levels are regulated by the N-end rule pathway (Hu et al. 2005, Lee et al. 2005). Recent data showed that NO is required for Cys residue oxidation in N-terminal motif of RGS proteins prior ubiquitin-dependent proteosomal degradation (Hu et al. 2005). Thus, NO may play a major role in regulating myocyte hypertrophy by controlling myocyte RGS protein levels. In this scheme of things low NO levels favor RGSs stabilization by preventing ubiquitin-dependent degradation and thereby blunting Gq-mediated signal, while high NO levels favor RGSs ubiquitination and derepression of the hypertrophic program (Figure 1, right

side). This new link between NO and RGS ubiquitin-dependent proteolytic pathway opens up a new perspective on the role of NO in cardiac hypertrophy which may change the actual understanding of anti vs. pro-hypertrophic effect.

Cardiomyocyte-dependent pro-angiogenic stimulation: a potential feedback loop

It is now well accepted that angiogenesis and VEGF signaling pathway are important for physiological cardiac hypertrophy whereas the reduction of capillary bed hallmarks the transition from compensated to uncompensated heart failure. In pressure overload experiments, VEGF trap administration accelerates heart failure progression presumably due to inhibition of compensatory growth of the vascular bed or, possibly, reduction in the size of the existing vasculature (Izumiya et al. 2006).

Nevertheless, the coupling of coronary angiogenesis with myocyte growth is essential for physiological cardiac hypertrophy as well (Walsh and Shiojima 2007). This paradigm is strengthened by the evidences of myocyte-dependent stimulation of cardiac angiogenesis. The two key factors thought to control the heart growth are serine-threonine kinase Akt1 and nutritional status (Shiojima et al. 2002). A short-term activation (2 weeks) of an inducible cardiac-specific Akt1 generates a physiological form of hypertrophy that is accompanied by myocardial angiogenesis secondary to myocyte-secreted VEGF and Ang-2. The latter events are induced via the Akt/mTOR pathway (Shiojima et al. 2005).

However, Akt1 activation for longer periods of time results in disproportional hypertrophy to the extent of angiogenesis eventually leading to heart failure. This suggests that angiogenic adaptation is not limitless and that it needs to be closely matched to the extent of hypertrophic response in order to maintain normal cardiac function.

In addition to activating VEGF and Ang2 production, Akt overexpression also induces expression of follistatin-like 1 factor (Fstl1), a secreted cardioprotective factor (Oshima et al. 2008). Fstl1 functions as an antiapoptotic protein and may be involved in adaptive hypertrophic response following pressure-overload and myocardial infarction (Oshima et al. 2008). Interestingly, Fstl1 expression in skeletal muscle in the setting of hindlimb ischemia promotes revascularization and induces activation of Akt-eNOS-dependent signaling in endothelial cells (Ouchi et al. 2008).

Fstl1 is a distantly related homolog of follistatin which has been shown to mediate myoblast fusion during prenatal and adult myogenesis. Recently data showed that NO directly stimulates myoblast fusion through the up-regulation of follistatin (Pisconti et al. 2006). NO effect is limited to fusion process itself and is mediated by guanylate cyclase activation and generation of cGMP.

Another example of myocyte proangiogenic stimulation is revealed by GATA4, a transcription factor that modulates cardiomyocyte differentiation and adaptive response in adult heart. Conditional GATA4 overexpression in the heart induced an increase of capillary and small artery densities by enhancing VEGF-A transcription (Heineke et al. 2007).

Summary

The two related themes that have emerged as a result of recent investigations are a multifactorial and cell-type specific activity of NO and cell-cell interactions in the myocardium that control its growth, size and function. NO is able to affect various cell types, in each case generating different functional results (Figure 2). Thus, secretion of NO by the endothelium, myocytes

and other cell types also affects cardiac tissues in a very complex manner. At the same time, it has become increasingly clear that all of the key cardiac cell types involved in regulation of myocardial function as well as extracardiac cells such as adipocytes may affect cardiac function via secretion of bioactive molecules (Figure 3). Endothelium in particular appears to play a key role in regulation of tissue and organ growth and size (Tirziu and Simons 2008).

The evidences of endothelium-induced myocardial hypertrophy in the absence of any traditional stimuli points to the existence of previously unappreciated cardiac growth regulatory mechanisms. In part this is mediated via NO, but other (unknown) factors are clearly also involved. The role of NO as a growth modulator is dependent on its autocrine and paracrine effects. NO supports angiogenesis in the autocrine fashion and mediates cardiomyocyte hypertrophy and vessel dilation via a paracrine signal (Figure 2). However, despite the large amount of data, the role of NO in regulation of cardiac hypertrophy is still uncertain.

Conceptually, a growth event in the heart requires an orchestrated action of multiple factors. A proportional growth of vasculature and cardiomyocytes is needed to preserve cardiac function both during development and in adult heart when hypertrophy is induced secondary to a mechanical stimulus. In order to keep this balanced growth of the myocardium and its vasculature, an integrative growth signal must be generated. However, the nature of this signal (s) and how it is generated are not known. It is likely that an active cross-talk at the level of cytokines and other bioactive molecules such as NO, secreted by both cardiac myocytes and cardiac endothelial cells, is involved. Cytokines and NO released by the endothelium (endokines) may stimulate myocyte growth. Reciprocally, cytokines released by myocytes (myokines) stimulate angiogenesis and may activate endothelium. In addition, cytokines released by other cell types including cardiac nerves (neurokines) and adipose tissue (adipokines) may also affect both the endothelium and cardiac myocytes adding another level of complexity to integration of the growth response (Figure 3). As these concepts begin to emerge, much will need to be done to understand complex interconnected growth signals involved in regulation of cardiac growth and hypertrophy.

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Figure 1.

NO-mediated endothelial paracrine signal. NO released by the endothelium as result of eNOS activation has a vasodilatation effect on the vasculature and cardiomyocytes and dual effect on cardiomyocyte hypertrophy. Left side: NO inhibits myocyte hypertrophy via PKG-induced suppression of NFAT-transcriptional activity. PKG activation reduces Ca²⁺ availability and calcineurin activity by inhibiting L-type Ca²⁺ channels thus preventing NFAT translocation in nucleus and hypertrophic gene program induction. Right side: NO increases ubiquitin-dependent proteosomal degradation of negative regulators of G-proteins thus sustains the propagation of hypertrophic stimulation via activated Gαq. Cytoplasmic levels of RGS proteins is controlled by NO through N-end rule pathway. High NO levels favor RGSs ubiquitination and maintain Gα-subunit in active form. Gα activates PLCβ which in turn generates DAG and IP₃. The released DAG activates PKC to initiate protein kinase cascade, increases Ca²⁺ intracellular influx and finally induces hypertrophic gene program by NFAT and MAPK pathways. Released IP₃ interacts with IP₃-receptors that cause a localized Ca²⁺ response resulting in activation of IP₃-R-associated CAMK. CAMK phosphorylates HDAC4/5 and facilitates their nuclear exit, and disinhibits growth-related gene transcription. sGCs –soluble guanylyl cyclases; GPCR - G-protein-coupled receptor; αq, β, γ -subunits of G protein heterotrimeric complex; PKG - cGMP-dependent protein kinases; DAG - diacylglycerol; IP₃ – inositol trisphosphate; PLC – phospholipase C; PKC – phosphokinase C; RGS – regulatory of G-protein signaling; NFAT – nuclear factor activated T cell; MAPK – mitogen activated protein kinases; HDAC – histone deacetylases; CAMK – calmodulin-dependent protein kinase; Ub – ubiquitin.

Figure 2.

Multifactorial effects of nitric oxide. NO exerts different effects on different cell types. Endothelial cells: acts as an autocrine factor supporting angiogenesis and increase in endothelial cell mass. The latter further raises NO production which in turn may induce cardiac hypertrophy. Cardiomyocytes: NO induces myocyte hypertrophy in a paracrine manner. The exact molecular mechanism is not known but may be related to Gq/Gi protein signaling activation as result of reduced RGS availability caused by increased NO levels. Smooth muscle cells: a paracrine NO-mediated signal in smooth muscle cells leads to vasodilation which in turn reduces preload/afterload diminishing hypertrophy induction. EC- endothelial cell; SMC-smooth muscle cell; CM-cardiomyocyte.

Figure 3. Cellular cross-talk. Each of the cardiac cell types and extracardiac adipocytes has the ability to release bioactive molecules that affect other cells types in paracrine and/or autocrine manner.