Cross talking molecular mechanisms in the failing heart: present and future targets of medical therapy

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The old heart failure (HF) treatments, which increased contractility, generally also increased mortality, whereas neurohumoral antagonism has successfully improved survival. Nevertheless death rate and disability remain major social problems, mainly for patients undergoing treatment, either due to late treatment initiation, inappropriate dosages or lack of persistence owing to possible side effects. This brief review will address the neurohumoral overdrive that makes the heart fail and triggers most, if not all, the changes in molecular mechanisms recently demonstrated to occur in HF syndrome. This review will also draw the attention of cardiologists to the suggestions for guidelines as to prescribing drugs that restore normal adrenergic receptors' density, thus providing a simultaneous improvement in other interdependent mechanisms.

Molecular mechanisms related to the increase in circulating neurohormones; some exciting novel heart failure therapies, such as replacement of the ischaemic or failing cells with new cardiomyocytes from stem cells; and the changes of adrenergic signalling by selectively modulating specificmolecules, will also briefly be taken into account.

Heart failure (HF) is the final pathway for most of the diseases that affect the heart. In recent decades we have observed an unprecedented improvement in mortality and morbidity due to the widespread use of coronary artery bypass graft, thrombolysis in myocardial infarction (TIMI) and percutaneous coronary interventions (PCI) in association with medical treatment.[1] Nevertheless, despite full revascularisation of the epicardial coronary arteries, HF is still one of the major causes of death in the population over 65 years of age, since we still lack the full knowledge of the myriads of molecular mechanisms responsible for HF progression.

A recent report shows that the improvement in long-term mortality in elderly patients after myocardial infarction is mainly due to the increased use of cardiovascular medication.[2]

This brief review will focus on some of the molecular mechanisms interrelated with the sympathetic nervous system drive and has the scope of drawing the attention of cardiologists to the guideline suggestions, in order to induce patients to follow the prescriptions and tolerate the possible initial side effects.[1] In addition some details will be given on potential future fields of investigation or potential therapies that might add to the already vast association of useful drugs.

MYOCARDIAL REMODELLING

Myocardial remodelling indicates the structural changes of the ventricular shape by abnormal loading conditions that become deleterious to the overall cardiac function. Volume and pressure overload lead to cell death by apoptosis and fibrosis. The transition from apparently compensated hypertrophy towards negative LV remodelling affects myocytes and the extracellular matrix by different cellular mechanisms, which primarily reduce the negative pressure gradient and ventricular diastolic suction. Ventricular suction is the vital process that allows rapid ventricular filling at low pressures.[3] Most of the approaches to treatment of recent decades were aimed to counteract pressure and volume overload. Profibrotic neurohumoral responses, together with increased levels of circulating neurohormones,[4] reactive oxygen species (ROS), and a changing balance between metalloproteinases and their inhibitors; and potentially genetic factors are the major molecular mechanisms implicated in the progression of heart failure.[4]

After myocardial infarction, the necrotic cells lose their physiological contractile properties, but the abnormal loading conditions involve not only the ischaemic myocardium but also the border zones of the infarcted area, and, amazingly, also the remote myocardium. In the clinical setting, in contrast with the animal model in which the occlusion of one major vessel is immediately followed by a diffuse 'compensatory' vasodilatation,[5] a relevant vasoconstriction takes place in infarcted and in remote regions, irrespective of prompt reperfusion of the occluded vessel.[6,7] In the clinical setting this vasoconstriction - and consequently the reduction in LV shortening - is induced by multiple factors[6–10] including ischaemia, necrosis, apoptosis, inflammation,[8] ROS formation, myocytes slippage and distal microembolisation.[9]

It is likely that the increase in circulating neurohormones plays a major role. In our clinical observations,[6,10,11] the intracoronary administration of α-adrenergic blockers counteracted LV dysfunction and vasoconstriction. When α-adrenergic blockers were additionally given on top of adenosine, the coronary flow reserve was improved.[12] Previous animal studies have shown that distal microsphere embolisation is followed by adenosine release by the non-embolised neighbouring vessels. Accordingly also the flow improvement seen after PCI and interpreted as postdilatation hyperaemia may be due to distal embolisation.[9,12,13]

NEUROHUMORAL ACTIVATION

Both the adrenergic and the renin-angiotensin-aldosterone systems are activated by ventricular dilation and are responsible for heart failure.

Up- and down-regulation of adrenergic-receptor density

Adrenergic receptors (ARs) exist in the myocardium and mediate many of the physiological responses of heart function via stimulation by the sympathetic transmitters, norepinephrine and epinephrine.[14] Adrenergic receptors are

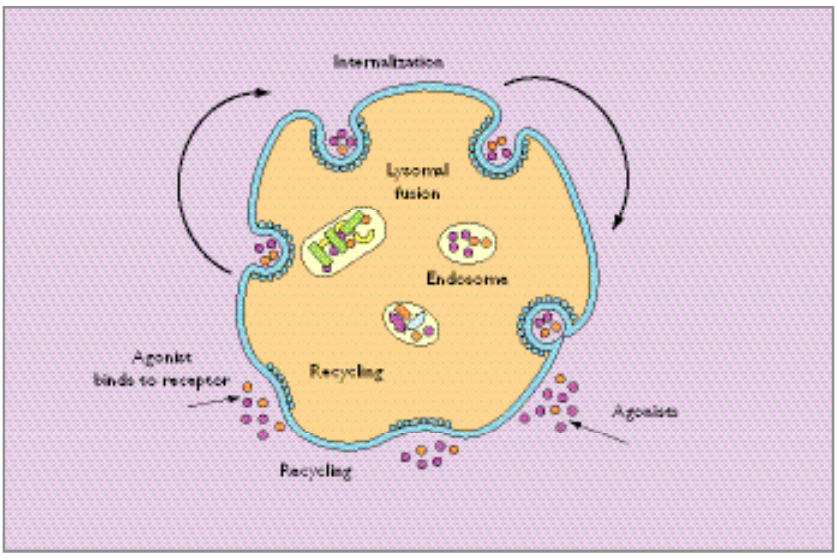

Figure 1. Shows a simplified schema of the dynamic of an adrenergic receptor and of an agonist action. Agonist binds to externalised receptors to give rise to cell reactions. Then receptors aggregates, bind to agonist, internalise and recycle returning on the cell surface. The lack of recycling and re-externalisation alters cell homeostasis and function, as it occurs in HF or in the presence of ischaemia.

divided into two principal types, alpha- and beta-receptors, which possess opposite but 'cross-talking' effects.[14–16] Many subtypes were identified following their response to chemicals or drugs and are extensively described elsewhere.[15] In brief, nine subtypes, three subtypes each of α1-, α2-, and ß-adrenergic receptors were described.[15] Each type preferentially links to members of a subfamily of G protein. The number of activated G proteins exceeds the number of corresponding receptors and effectors; thus, the activation of G proteins amplifies signalling by the adrenergic receptors.[17] Receptors' desensitisation, mediated in part by G-protein–receptor kinases and ß-arrestins, is involved in decreasing the ability of agonists to activate adrenergic receptors.[17] In heart failure, adrenergic receptors progressively desensitise and, accordingly, the contracting capacity of myocytes decreases.[18,19] Persistent exposure to agonists can also result in an actual loss of receptors, presumably through degradation, but this type of receptor down-regulation occurs more slowly. The susceptibility to these agonist-promoted events differs among the various types and subtypes of adrenergic receptors.[18,19]

The downregulation of adrenoceptor density in human HF is a progressive mechanism that in the advanced HF stages becomes irreversible, whereas in the animal model in which HF is obtained by pacing, the adrenoceptor density returns to normal level by week four after cessation of pacing.[18,19] The pathophysiology of adrenoreceptors in ischaemia and in HF is still mainly unknown.

In the canine model it is possible to induce HF by multiple sequential coronary microembolisation.[20] Distal coronary embolisation is likely to be the major factor that limits the results obtained after TIMI[7], PCI[12] and PAMI.[9]

Adrenergic receptor alterations in the clinical setting

The knowledge of receptors endocytosis, i.e., the receptor-mediated mechanism used by cells for the uptake of macromolecules from the extracellular fluid, has recently deflected the focus on adrenergic receptors that internalise to elicit a cellular function and subsequently externalise to be ready to initiate a second endocytosis (Figure 1).[21] An impairment of cardiac norepinephrine reuptake through neuronal norepinephrine transporter promotes depletion of cardiac norepinephrine stores and local cardiac sympathetic activation in myocardial ischaemia and in heart failure.[22]

In fact ß-adrenergic receptor kinases (ß ARK1) are elevated in the failing heart and in myocardial ischaemia likely to be triggered by heightened sympathetic nervous system activity.[22,23] It is demonstrated that also the alterations in myocardial ß-AR signalling present in myocardial ischaemia mirror those found in chronic HF.[24]

It is now well established that up- and down-regulation of the receptors - and consequently their availability - play a key role in cardiomyocytes contraction and in the homeostasis of coronary artery tone.[22–25] In particular, in hibernating cardiomyocytes, the adrenergic receptors' density changes following the severity of ischaemia.[26] In fact, it was shown that the density of α-adrenoreceptors progressively increases in hibernating and non-functional myocardium, whereas, in contrast, ß-adrenoreceptors decrease in dysfunctional myocardium.[26]

The adrenergic-mediated attenuation of resting myocardial function was also demonstrated in experimental models of myocardial ischaemia.[27–28]

Receptors may be targeted to the lysosome for degradation or recycled back to the cell surface for further rounds of uptake.[21] Recent developments using the techniques of site-directed mutagenesis, cell-free assays and cell fractionation have begun to elucidate the endocytic mechanism, its dynamics and regulation.[27–29]

When functional recovery of receptors is absent, as in ischaemia or in HF, the myocyte contraction and the response to inotropic drugs does not occur. Evidence from both neural recordings and measurement of circulating catecholamines clearly documents a central origin for much of the sympathetic activation in chronic HF.[30,32] In HF there is also an impairment of cardiac norepinephrine reuptake through the neuronal norepinephrine transporter that promotes depletion of cardiac norepinephrine stores and local cardiac sympathetic activation (Figure 2).[30–32]

The mechanisms involved in sympathetic dysfunction appear to be, in these disorders, multifactorial.[4,30–32] Alterations in the local production of substances such as bradykinin, NO, prostaglandins,[33] and so forth, have all been implicated as excitatory substances for cardiac and chemoreceptor afferents in heart failure. The sympathetic nerve function is not only regulated by the central nervous system but also by the target organ. Patients with congestive heart failure have a chronic and increased activation of the sympathetic nervous system that induces a progressive worsening of left ventricular dysfunction, and cardiac remodelling.

Genetic causes and genes polymorphism

The response of the heart to overload or injury is the activation of intracellular signalling pathways that elicits heart-failure phenotype that in turn induces myocyte hypertrophy, re-expression of an embryonic gene pattern, including up-regulation of structural fetal genes, such as beta-myosin heavy chain, and down-regulation of adult structural genes, such as a-myosin heavy chain, and remodelling of the extracellular matrix. These events lead to left ventricular negative remodelling and progressive myocardial fibrosis.[34]

Activation of the renin-angiotensin-aldosterone systems

Ventricular overloading also activates the renin-angiotensin-aldosterone system.[33,35] Angiotensin II is synthesised directly within the myocardium; probably exaggerates the activation of metalloproteinases,[36] apop-

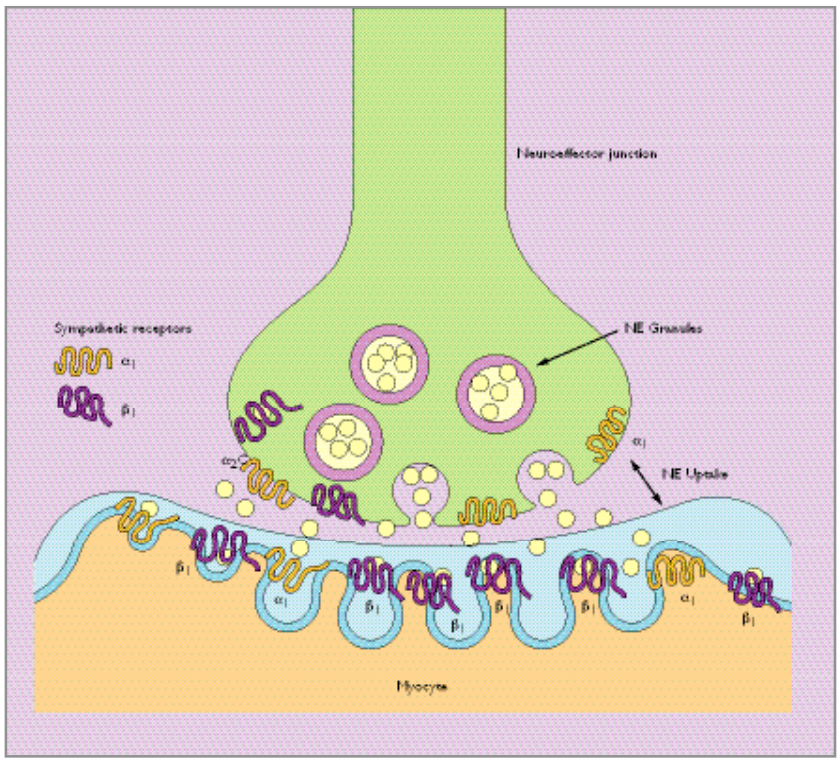

Figure 2. Schema of sympathetic nerve receptors that regulate the synaptic release of norepinephrine at a neuroeffector junction. The autofeedback loop that regulates the synaptic reuptake of norepinephrine (NE) is altered in advanced HF, and myocyte function is impaired.

totic cardiomyocyte death and increased cytosolic calcium; and promotes the expression of the profibrotic mediator, transforming growth factors -β (TGF-β) expression, thus further increasing mechanical dysfunction.[37,38] Moreover, increased myocardial angiotensin II augments apoptosis in ventricular myocytes, also by ROS formation.[8] Conversely, angiotensin-converting-enzyme (ACE) inhibition blocks TGF-β and lessens myocardial and perivascular fibrosis.[38] Increased angiotensin-II-mediated release of aldosterone promotes both sodium retention and cardiac fibrosis.[39] The combination of fibrosis and increased cell death leads to a disorganised, poorly contracting myocardium. [40]

ROLE OF EXTRACELLULAR MYOCARDIAL MATRIX

The myocardial extracellular matrix (ECM) was once considered a static structure. Now it is known to be a complex microenvironment containing a large portfolio of matrix proteins, signalling molecules, proteases and cell types that play a fundamental role in the myocardial remodelling process.[41]

ECM is synthesised by cardiac fibroblasts[42,43] and maintains myocyte orientations throughout the LV free wall.[41] The collagen synthesis is modulated by metalloproteinase and its inhibitors. When matrix metalloproteinases inhibitors increase activity, collagen crosslinks degenerate so that the hypertrophied left ventricle begins to dilate and causes fibrosis. ECM maintains alignment of myofibrils within the myocyte through a collagen-integrin-cytoskeleton-myofibril relation. In addition to a fibrillar collagen network, a basement membrane, proteoglycans, and glycosaminoglycans, the myocardial ECM contains a large reservoir of bioactive molecules.[41] It has been demonstrated that the concentration of bioactive signalling molecules such as angiotensin II (ANG II) and endothelin -1 (ET-1) are over 100-fold higher within the myocardial interstitium than in plasma.[41] Moreover, cytokine activation and signalling - such as that for tumour necrosis factor-α (TNF-α) - is highly compartmentalised within the myocardial interstitium. Growth factors such as TGF-β are stored in a latent form within the myocardial interstitium and thereby form a reservoir of signalling molecules that directly influence myocardial ECM synthesis and degradation.

CONTROL ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN ACTIVE MOLECULES OVEREXPRESSION

Increased overexpression of biologically active molecules that are capable of exerting deleterious effects on the heart and circulation, such as angiotensin II, endothelin, natriuretic peptides and TNF-α, are peptide growth factors and/or cytokines that are produced by a variety of cell types within the heart, including cardiac myocytes.[41–42] Despite the fact that biologically active molecules do not have a neuroendocrine origin, they are likely to be under the control of neurohormonal activity.[43]

ROLE OF β-BLOCKERS IN HEART FAILURE

Beta-adrenergic blockers have a remarkable effect on the progression of HF, primarily counteracting the deleterious increase of the sympathetic drive that downregulates adrenergic receptors, which are essential for myocytes contraction. Beta-blockers have a direct beneficial effect on the myocardium, enhancing reverse remodelling. Selected agents that also block the α-adrenergic receptors induce vasodilatation.[44–48] The beneficial effects of these drugs include improvements in survival, morbidity, ejection fraction, remodelling, quality of life, the rate of hospitalisation and the incidence of sudden death. Beta-blockers should be used in all patients in stable condition without substantial fluid retention and without recent exacerbations of heart failure requiring inotropic therapy. Although the short-term effects of β-blockers may result in a temporary exacerbation of symptoms, their long-term effects are uniformly beneficial.

ACE INHIBITORS AND ALDOSTERONE ANTAGONISTS

Angiotensin-converting–enzyme (ACE) inhibitors and angiotensin-receptor blockers decrease afterload by interfering with the renin–angiotensin–aldosterone system, resulting in peripheral vasodilatation. They also affect left ventricular hypertrophy, remodelling and renal blood flow. Aldosterone antagonists counteract the effects of aldosterone.

Attenuation or even reversal of left ventricular dilatation after myocardial infarction and decrease of the incidence of recurrent myocardial infarction in patients with coronary artery disease was an unexpected finding. Such beneficial effects were associated with so-called reverse remodelling, in which the therapy promoted a return to a more normal ventricular size and shape. The reverse-remodelling process is a mechanism through which a variety of treatments palliate the heart-failure syndrome.[49,50]

STATINS

Simvastatin has been shown to be effective in lowering the incidence of new-onset heart failure, and improving HF outcome.[51] Favourable statins effects may be a result of both lipid and nonlipid mechanisms affecting endothelial vasoregulation, and normalisation of sympathetic outflow[52] that induces pronounced peripheral vasoconstriction, sodium retention, attenuated cardiovascular reflexes, and susceptibility to ventricular arrhythmias and sudden cardiac death. Statins are shown to be neuroprotective, in part, by a nitric oxide (NO)-dependent mechanism[53] and

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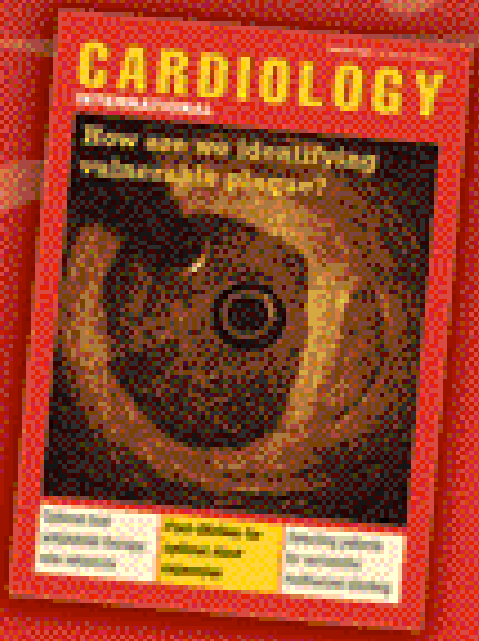

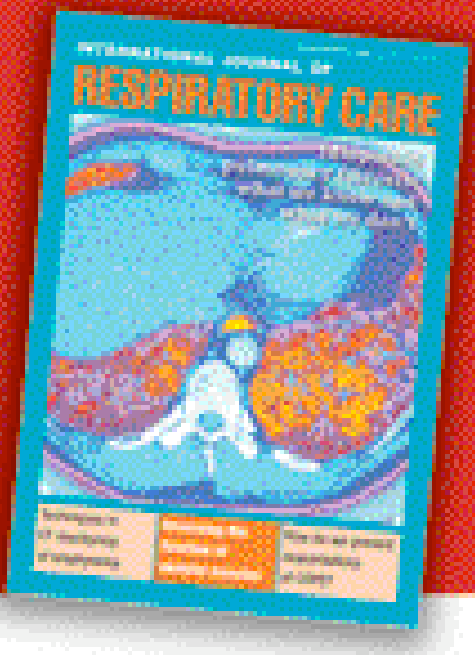

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in part because NO is sympathoinhibitory, and accordingly lower sympathetic outflow.[54, 55]

INFLAMMATION

Inflammatory response and cytokine elaboration are particularly active after myocardial infarction[8] and contribute to survival or deaths of myocytes. TNF-α or interleukin-6 acutely regulate myocyte survival or apoptosis and trigger additional cellular inflammatory response. The triggers of cytokine release include mechanical deformation, ischaemic stimulus, ROS and cytokine self-amplification pathways. Chronically, the presence of cytokines leads to myocyte phenotype transition and activation of matrix metalloproteinases that modifies interstitial matrix, and increases the remodelling process. These processes may induce angiogenesis and cellular regeneration.[44] Thus, the modulation of cytokines through current and future therapies could promote improved healing and cardiac remodelling.

PROTECTIVE ROLE OF CYCLIC GMP—SILDENAFIL AS POTENTIAL TREATMENT OPTION

The inhibitory cardiovascular signals mediated by cyclic guanosine monophosphate (GMP) modulate adrenergic effects.[56] Furthermore, genetically increased synthesis of cyclic GMP inhibits pressure-induced pathological remodelling.[57] Sildenafil inhibits the breakdown of cyclic GMP and neutralises many growth pathways to prevent and reverse hypertrophy and failure induced by aortic constriction.[57] By cyclic-GMP-induced formation of nitric oxide, sildenafil might reduce vascular stiffness acting as a systemic vasodilator in patients with heart failure.[58]

THE FUTURE: SEARCH FOR A BETTER TREATMENT

Stem cells

Many clinical problems that occur in patients with heart failure remain unsolved. A better knowledge of the molecular mechanisms will improve treatment of the HF syndrome. Recently the concept of the heart as a post-mitotic organ was superseded by the notion of the heart as a self-renewing organ regulated by multipotent cardiac stem cells capable of regenerating myocytes and coronary vessels throughout life.[59] In the past decade, numerous stem cell studies have shown extrinsic and intrinsic regeneration of myocytes and coronary vessels. Cardiac stem cell therapy may become, perhaps in the near future, a novel strategy for the social problem of heart failure.[60]

Alteration of myocardial adrenergic receptor signalling to modulate in-vivo cardiac function

Genetically engineered mouse models have recently provided the knowledge that in-vivo contractility can be enhanced via alteration of myocardial adrenergic receptor (AR) signalling.[61] Thus genetic manipulation of the AR signalling system in the heart, including its regulation by desensitising kinases, represents a novel therapeutic approach for improving function of the failing heart. In addition to a gene therapy approach, the discovery of small molecules to inhibit β ARK1 activity could lead to novel drugs that might prove more selective in improving performance of the failing heart. [62]

Cardiac gene delivery

Recent preliminary advances in cardiac gene delivery have been reported in animal models.[63] In this context, gene transfer provides a potential therapeutic modality. Several interventions, particularly those enhancing sarcoplasmic calcium transport, show promise in animal models of heart failure and in myopathic cardiomyocytes derived from patients. However, the gap between the basic studies and clinical gene therapy remains far from being clarified and early experiments need to be extended to large-animal models to assess both efficacy and safety. Only time and further research will tell if these can be successfully extended to clinical practice.

REFERENCES

1. Hunt SA, Abraham WT, Chin MH *et al.* ACC/AHA 2005 Guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force. *Circulation* 2005; **112**: e154–e235.
2. Setoguchi S, Glynn RJ, Avorn J, Mittleman MA, Levin R, Winkelmayer WC. Improvements in long-term mortality after myocardial infarction and increased use of cardiovascular drugs after discharge: A 10-year trend analysis. *J Am Coll Cardiol* 2008; **51**: 1247–1254.
3. Sanderson JE. Left and right ventricular long-axis function and prognosis. *Heart* 2008; **94**: 262–263.
4. Zucker IH, Wang W, Brändle M, Schultz HD, Patel KP. Neural regulation of sympathetic nerve activity in heart failure. *Prog Cardiovas Dis* 1995; **37**: 397–414.
5. Gould KL, Lipscomb K, Calvert C. Compensatory changes of the distal coronary vascular bed during progressive coronary constriction. *Circulation* 1975; **51**: 1085–1094.
6. Gregorini L, Marco J, Kozàkovà M *et al.* a–Adrenergic blockade improves recovery of myocardial perfusion and function after coronary stenting in patients with acute myocardial infarction. *Circulation* 1999; **99**: 482–490.
7. Gibson CM, Ryan KA, Murphy SA *et al.* for the TIMI Study Group. Impaired coronary blood flow in nonculprit arteries in the setting of acute myocardial infarction. *J Am Coll Cardiol* 1999; **34**: 974–982.
8. Nian M, Lee P, Khaper N, Liu P. Inflammatory cytokines and postmyocardial infarction remodelling. *Circ Res* 2004; **94**: 1543–1553.
9. Bahrmann P, Werner GS, Heusch G *et al.* Detection of coronary microembolization by Doppler ultrasound in patients with stable angina pectoris undergoing elective percutaneous coronary interventions. *Circulation* 2007; **115**: 600–608.
10. Gregorini L, Fajadet J, Robert G, Cassagneau B, Bernis M, Marco J. Coronary vasoconstriction following percutaneous transluminal coronary angioplasty is attenuated by antiadrenergic agents. *Circulation* 1994; **90**: 895–907.
11. Gregorini L, Marco J, Palombo C *et al.* Post-ischemic left ventricular dysfunction is abolished by alpha-adrenergic blockers. *J Am Coll Cardiol* 1998, **31**: 992–1001.
12. Gregorini L, Marco J, Farah B *et al.* Effects of selective a1 and a2–adrenergic blockade on coronary flow reserve after coronary stenting. *Circulation* 2002; **106**: 2901–2907.
13. Hori M, Inoue M, Kitakaze M *et al.* Role of adenosine in hyperemic response of coronary blood flow in microembolization. *Am J Physiol* 1986; **250**: H509–H518.
14. Lefkowitz RJ, Caron MG, Stiles GL. Mechanisms of membrane-receptor regulation: biochemical, physiological, and clinical insights derived from studies of the adrenergic receptors. *N Engl J Med* 1984; **310**: 1570–1579.
15. Bylund DB, Eikenberg DC, Hieble JP *et al.* International union of pharmacology nomenclature of adrenoceptors. *Pharmacol Rev* 1994; **46**: 121–136.
16. Akhter SA, Milano CA, Shotwell KF *et al.* Transgenic mice with cardiac overexpression of alpha1B-adrenergic receptors: in vivo alpha1-adrenergic receptor-mediated regulation of beta-adrenergic signaling. *J Biol Chem* 1997; **272**: 21253–21259.
17. Insel PA. Adrenergic receptors – evolving concepts and clinical implications. *N Engl J Med* 1996: **334**: 580–585.
18. Kiuchi K, Shannon RP, Komamura K *et al.* Myocardial beta-adrenergic receptor function during the development of pacing-induced heart failure. *J Clin Invest* 1993; **91**: 907–914.
19. Kawai H, Mohan A, Hagen J *et al.* Alterations in cardiac adrenergic terminal function and beta-adrenoceptor density in pacing-induced heart failure. *Am J Physiol* 2000; **278**, H1708–H1716.
20. Sabbah NH, Stein PD, Kono T *et al.* A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. *Am J Physiol* 1991; **260**: H1379–H1384.

21. Hollenberg MD. Structure-activity relationships for transmembrane signaling: the receptor's turn. *FASEB J* 1991; **5**: 178–186.
22. Ungerer M, Bohm M, Elce JS, Erdmann E, Lohse MJ. Altered expression of beta-adrenergic receptor kinase and beta1–adrenergic receptors in the failing human heart. *Circulation* 1993; **87**: 454–463.
23. Zucker I H. Novel mechanisms of sympathetic regulation in chronic heart failure. *Hypertension* 2006; **48**: 1005–1011.
24. Bristow MR, Hershberger RE, Port JD *et al.* Beta–adrenergic pathways in nonfailing and failing human ventricular myocardium. *Circulation* 1990; **82**: Suppl I: I–12.
25. Bristow MR, Minobe WA, Raynolds MV *et al.* Reduced beta 1 receptor messenger RNA abundance in the failing human heart. *J Clin Invest* 1993; **92**: 2737–2745.
26. Shan K, Bick RJ, Poindexter BJ *et al.* Altered adrenergic receptor density in myocardial hibernation in humans. a possible mechanism of depressed myocardial function. *Circulation* 2000; **102**: 2599–2606.
27. Zhao M, Hagler HK, Muntz KH. Regulation of alpha 1–, beta 1–, and beta 2–adrenergic receptors in rat heart by norepinephrine. *Am J Physiol* 1996; **271**: H1762–H1768.
28. Grupp IL, Lorenz JN, Walsh RA *et al.* Overexpression of alpha1B–adrenergic receptor induces left ventricular dysfunction in the absence of hypertrophy. *Am J Physiol* 1998; **275**: H1338–H1350.
29. Tamagawa K, Saito T, Oikawa Y, Maehara K, Yaoita H, Maruyama Y. Alterations of a–adrenergic modulations of coronary microvascular tone in dogs with heart failure. *J Card Fail* 2005; **11**: 388–395.
30. Schmid PG, Whiteis CA, Lund DD. Ventricular hypertrophy and presynaptic regulation of sympathetic function. *Am J Physiol* 1990; **258**: H1375–1381.
31. Aggarwal A, Esler MD, Lambert GW, Hastings J, Johnston L, Kaye DM. Norepinephrine turnover is increased in suprabulbar subcortical brain regions and is related to whole-body sympathetic activity in human heart failure. *Circulation* 2002; **105**: 1031–1033.
32. Mann DL, Bristow MR. Mechanisms and models in heart failure: the biomechanical model and beyond. *Circulation* 2005; **111**: 2837–2849.
33. Dzau VJ, Packer M, Lilly LS, Swartz SL, Hollenberg NK, Williams GH. Prostaglandins in severe congestive heart failure: relation to activation of the renin-angiotensin system and hyponatremia. *N Engl J Med* 1984; **310**: 347–352.
34. Thum T, Galuppo P, Wolf C *et al.* MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. *Circulation* 2007; **116**: 258–267.
35. Dzau VJ, Colucci WS, Hollenberg NK, Williams GH. Relation of the renin–angiotensin-aldosterone system to clinical state in congestive heart failure. *Circulation* 1981; **63**: 645–651.
36. Banfi C, Cavalca V, Veglia F *et al.* Neurohormonal activation is associated with increased levels of plasma matrix metalloproteinase–2 in human heart failure. *Eur Heart J* 2005; **26**: 481–488.
37. Katada J, Meguro T, Saito H *et al.* Persistent cardiac aldosterone synthesis in angiotensin II type 1A receptor–knockout mice after myocardial infarction, *Circulation* 2005; **111**: 2157–2164.
38. Peng H, Carretero OA, Vuljaj N *et al.* Angiotensin-converting enzyme inhibitors: a new mechanism of action. *Circulation* 2005; **112**: 2436–2445.
39. Weber KT. Aldosterone in congestive heart failure. *N Engl J Med* 2001; **345**: 1689–1697.
40. Boluyt MO, O'Neill L, Meredith AL *et al.* Alterations in cardiac gene expression during the transition from stable hypertrophy to heart failure. Marked upregulation of genes encoding extracellular matrix components. *Circ Res* 1994; **75**: 23–32.
41. Spinale FG. Myocardial matrix remodelling and the matrix metalloproteinases: influence on cardiac form and function. *Physiol Rev* 2007; **87**: 1285–1342.
42. Polyakova V, Hein S, Kostin S, Ziegelhoeffer T, Schaper J. Matrix metalloproteinases and their tissue inhibitors in pressure-overloaded human myocardium during heart failure progression. *J Am Coll Cardiol* 2004; **44**: 1609–1618.
43. Banfi C, Cavalca V, Veglia F *et al.* Neurohormonal activation is associated with increased levels of plasma matrix metalloproteinase–2 in human heart failure. *Eur Heart J* 2005; **26**: 481–488.
44. Mann DL. Stress-activated cytokines and the heart: from adaptation to maladaptation. *Annu Rev Physiol* 2003; **65**: 81–101.
45. Bristow MR, Gilbert EM, Abraham WT *et al.* Carvedilol produces dose–related improvements in left ventricular function and survival in subjects with chronic heart failure. *Circulation* 1996; **94**: 2807–2816.
46. Packer M, Bristow MR, Cohn JN *et al.* The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. US Carvedilol Heart Failure Study Group. *New Engl J Med* 1996; **334**: 1349–1355.
47. Foody JM, Farrell MH, Krumholz HM. Beta-blocker therapy in heart failure: scientific review. *JAMA* 2002; **287**: 883–889.
48. Hall SA, Cigarroa CG, Marcoux L, Risser RC, Grayburn PA, Eichhorn EJ. Time course of improvement in left ventricular function, mass and geometry in patients with congestive heart failure treated with beta-adrenergic blockade. *J Am Coll Cardiol* 1995; **25**: 1154–1161.
49. Garg R, Yusuf S. Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure. *JAMA* 1995; **273**: 1450–1456.
50. The Heart Outcomes Prevention Evaluation Study Investigators. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000; **342**: 145–153.
51. Kjekshus J, Pedersen TR, Olsson AG *et al.* The effects of simvastatin on the incidence of heart failure in patients with coronary heart disease. *J Card Fail* 1997; **3**: 249–254.
52. Pliquett RU, Cornish KG, Peuler JD, Zucker IH. Simvastatin normalizes autonomic neural control in heart failure. *Circulation* 2003; **107**: 2493–2498.
53. Liu J-L, Zucker IH. Regulation of sympathetic nerve activity in heart failure: a role for nitric oxide and angiotensin II. *Circ Res* 1999; **84**: 417–423.
54. Gao L, Wang W, Li Y-L, Schultz HD, Liu D, Cornish KG, Zucker IH. Simvastatin therapy normalizes sympathetic neural control in experimental heart failure: Roles of angiotensin II type 1 receptors and NAD(P)H oxidase. *Circulation* 2005; **112**: 1763–1770.
55. Ramasubbu K, Mann DL. The emerging role of statins in the treatment of heart failure. *J Am Coll Cardiol* 2006; **47**: 342–344.
56. Borlaug BA, Melenovsky V, Marhin T, Fitzgerald P, Kass DA. Sildenafil inhibits beta-adrenergic-stimulated cardiac contractility in humans. *Circulation* 2005; **112**: 2642–2649.
57. Takimoto E, Champion HC, Li M *et al.* Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. *Nat Med* 2005; **11**: 214–222.
58. Hirata K, Adji A, Vlachopoulos C, O'Rourke MF. Effect of sildenafil on cardiac performance in patients with heart failure. *Am J Cardiol* 2005; **96**: 1436–1440.
59. Yoon Y-S, Wecker A, Heyd L *et al.* Clonally expanded novel angiotensin II multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction. *J Clin Invest* 2005; **115**: 326–338.
60. Shantsila E, Watson T, Lip GYH. Endothelial progenitor cells in cardiovascular disorders. *J Am Coll Cardiol* 2007; **49**: 741–752.
61. Koch WJ, LefkowitzRJ, Rockman HA. Functional consequences of altering myocardial adrenergic receptor signalling. *Annu Rev Physiol* 2000; **62**: 237–260.
62. Hata JA, Williams ML, Koch WJ, Genetic manipulation of myocardial b–adrenergic receptor activation and desensitization. *J Mol Cell Cardiol* 2004; **37**: 11–21.
63. Hajjar RJ, del Monte F, Matsui T, Rosenzweig A. Prospects for gene therapy for heart failure. *Circ Res* 2000; **86**: 616–621. n

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