

- 40 Tang-Christensen, M.T. *et al.* (2000) The proglucagon-derived peptide, glucagon-like peptide-2, is a neurotransmitter involved in the regulation of food intake. *Nat. Med.* 6, 802–807
- 41 Inui, A. (2000) Transgenic approach to the study of body weight regulation. *Pharmacol. Rev.* 52, 35–61
- 42 Collier, G.R. *et al.* (2000) Beacon: a novel gene involved in the regulation of energy balance. *Diabetes* 49, 1766–1771
- 43 Tschöp, M. *et al.* (2000) Ghrelin induces adiposity in rodents. *Nature* 407, 908–913
- 44 Stahl, A. *et al.* (1999) Identification of the major intestinal fatty acid transport protein. *Mol. Cell* 4, 299–308
- 45 Smith, S.J. *et al.* (2000) Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nat. Genet.* 25, 87–90
- 46 Lowell, B.B. (1998) Adaptive thermogenesis: turning on the heat. *Curr. Biol.* 8, R517–R520
- 47 Klingenberg, M. and Huang, S.G. (1999) Structure and function of the uncoupling protein from brown adipose tissue. *Biochim. Biophys. Acta* 1415, 271–296
- 48 Weyer, C. *et al.* (1999) Development of β -3 adrenoceptor agonists for the treatment of obesity – an update. *Diabetes Metab.* 25, 11–21
- 49 Rolfe, D.F.S. and Brown, G.C. (1997) Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* 77, 731–758
- 50 Boss, O. *et al.* (2000) Uncoupling protein 2 and 3: potential regulators of mitochondrial energy metabolism. *Diabetes* 49, 143–156
- 51 Schrauwen, P. *et al.* (1999) Skeletal muscle uncoupling protein 3 expression is a determinant of energy expenditure in Pima Indians. *Diabetes* 48, 146–149
- 52 Agropoulos, G. *et al.* (1998) Effects of mutations in the human uncoupling protein 3 gene on the respiratory quotient and fat oxidation in severe obesity and type 2 diabetes. *J. Clin. Invest.* 102, 1345–1351
- 53 Clapham, J.C. *et al.* (2000) Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. *Nature* 406, 415–418
- 54 Gong, D.W. *et al.* (2000) Lack of obesity and normal response to fasting and thyroid hormone in mice lacking uncoupling protein-3. *J. Biol. Chem.* 275, 16251–16257
- 55 Spiegelman, B.M. (1998) PPAR- γ : adipogenic regulator and thiazolidinedione receptor. *Diabetes* 47, 507–514
- 56 Kubota, N. *et al.* (1999) PPAR- γ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol. Cell* 4, 597–609
- 57 Anand, A. and Chada, K. (2000) *In vivo* modulation of Hmgic reduces obesity. *Nat. Genet.* 24, 377–380
- 58 Martinez-Botas, J. *et al.* (2000) Absence of perilipin results in leanness and reverses obesity in *Lepr^{db/db}* mice. *Nat. Genet.* 26, 474–479
- 59 Kim, J.K. *et al.* (2000) Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. *J. Biol. Chem.* 275, 8456–8460
- 60 Wu, Z. *et al.* (1999) Transcriptional activation of adipogenesis. *Curr. Opin. Cell Biol.* 11, 689–694

Chemical name

C75: *trans*-tetrahydro-3-methylene-2-oxo-5-*n*-octyl-4-furancarboxylic acid

Cardiac mechanotransduction: from sensing to disease and treatment

Pasi Tavi, Mika Laine, Matti Weckström and Heikki Ruskoaho

In heart muscle a mechanical stimulus is sensed and transformed into adaptive changes in cardiac function by a process called mechanotransduction. Adaptation of heart muscle to mechanical load consists of neurohumoral activation and growth, both of which decrease the initial load. Under prolonged overload this process becomes maladaptive, leading to the development of left ventricular hypertrophy and ultimately to heart failure. Widespread synergism and crosstalk among a variety of molecules and signals involved in hypertrophic signaling pathways make the prevention or treatment of left ventricular hypertrophy and heart failure a challenging task. Therapeutic strategies should include either a complete and continuous reduction of load or normalization of left ventricular mass by interventions aimed at specific targets involved in mechanotransduction.

Pasi Tavi*
Matti Weckström
Dept of Physiology and
Dept of Physical Sciences
(Division of Biophysics),
Biocenter Oulu
*e-mail: pasi.tavi@oulu.fi
Heikki Ruskoaho
Dept of Pharmacology and
Toxicology, Biocenter Oulu,
University of Oulu,
PO Box 5000, FIN-90014,
University of Oulu, Finland.
Mika Laine
Dept of Medicine, Helsinki
University Central
Hospital and Minerva
Institute, PO Box 341,
00029 Helsinki, Finland.

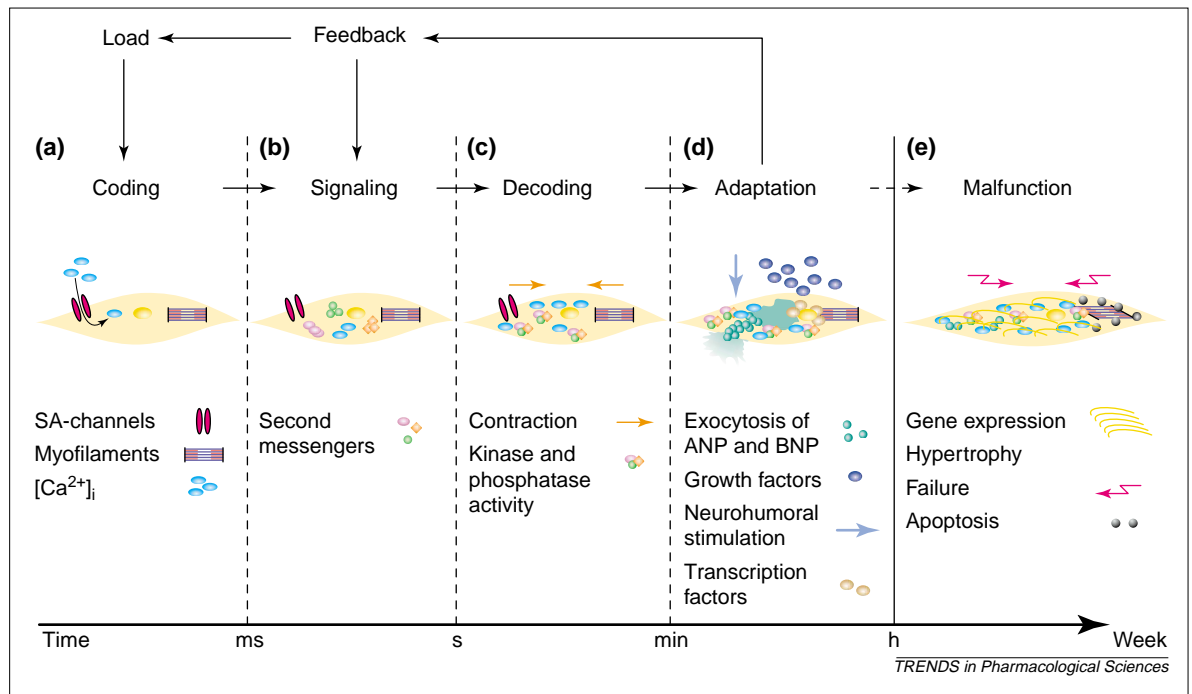
The process of sensing mechanical load and the consequent physiological responses is called mechanotransduction. In heart muscle, a mechanical stimulus is transformed into altered contraction force, altered ion balance, exocytosis or gene expression. In cardiac myocytes, mechanotransduction can be divided into a series of events, from the coding of the mechanical stimuli to second messengers and decoding the information into changes in heart function (Fig. 1). The same mechanosensors, signals, kinases and transcription factors are involved in both normal and pathological hypertrophy. Therefore, any given pharmacological intervention aimed at treating or preventing hypertrophy might influence not only pathological

developments but also normal adaptation and mechanotransduction itself. In this article, present and future pharmacological approaches will be discussed in the context of mechanotransduction.

Stretch-sensitive molecular elements in cardiac myocytes

In cardiac myocytes, Ca^{2+} defines contractile function but also serves as a second messenger that is able to control many other cellular functions (Figs 1,2). Therefore, it is not surprising that early events induced by mechanical stretch of cardiac muscle include an increase of contraction force, partly caused by an increase of the systolic Ca^{2+} transients¹. The nonselective cation channels that can be activated by longitudinal stretch of the cells² could be the possible stretch transducers. When opened, these channels cause an influx of Na^+ and Ca^{2+} , both of which are able to augment the Ca^{2+} transients via sarcoplasmic reticulum (SR) Ca^{2+} loading. Several agents such as aminoglycoside antibiotics and Gd^{3+} are known to inhibit these stretch-activated (SA)-channels but are relatively nonspecific. Indeed, the early events in mechanotransduction are inaccessible to pharmacological tools. For example, in isolated cardiac trabeculae even almost total inhibition of SR function with ryanodine and cyclopiazonic acid does

Fig. 1. Time-course of mechanotransduction in cardiac myocytes. (a) The increased load (e.g. stretch or distension) of the cardiac myocytes is coded by stretch-activated ion channels (SA-channels) and the contractile element. (b) This leads to signaling via various second messengers. (c) Decoding of these signals by kinases and phosphatases alters the function (contraction force) of myocytes and leads to (d) adaptational exocytosis and gene expression of atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). During this adaptational period several hormonal signals interact with the elements in myocytes that are involved in mechanotransduction. Physiologically, ANP and BNP fulfill the role of negative feedback control of mechanotransduction. Being vasorelaxant and natriuretic, ANP and BNP reduce the peripheral resistance and volume load, thus suppressing the stimuli leading to mechanotransduction. The feedback mechanisms also include neurohumoral activation and expression, and release of growth-promoting factors. The crucial events in the development of pathological hypertrophy might involve either suppression or enhancement of the adaptational mechanisms. (e) Transition from compensatory hypertrophy (adaptation) to heart failure includes complex changes in gene expression programming and apoptosis.



not inhibit the length-dependent changes in force and $[Ca^{2+}]_i$ (Ref. 3). However, the attachment of SA-channels to elements in the cytoskeleton (e.g. actin filaments) might offer a possibility for regulation, as shown in studies using fibroblasts⁴.

Perhaps the best known stretch sensor in cardiac myocytes is the contractile element (Fig. 1). Conformational changes in the interfilament spacing as a result of an increase of sarcomere length recruits more force-generating units (i.e. actin-myosin interactions). This fundamental feature of the contractile element enables the cardiac muscle to increase the contraction force following an increase of mechanical loading (i.e. the Frank-Starling behaviour). In addition, the Ca^{2+} -binding part of the contractile element, troponin C (TnC), exhibits increased Ca^{2+} affinity following an increase of the sarcomere length³. This sensitization results in an increase of the contraction force without changes in $[Ca^{2+}]_i$. Ca^{2+} sensitizers, such as levosimendan⁵, increase contraction force by sensitizing contractile proteins to Ca^{2+} without changes in $[Ca^{2+}]_i$, thus mimicking the effects of stretch on the TnC.

All effects of stretch or increased loading are not inherent to the cardiac myocytes. The release of autocrine and paracrine factors such as angiotensin II (Ang II) from fibroblasts and endothelin-1 (ET-1) from endothelial cells, triggered by cardiac wall stretch, might cause activation of additional pathways within cardiac myocytes^{6,7} (Fig. 2). Stimulation of endothelin ET_A receptors (a G_q -protein-coupled receptor) leads to activation of protein kinase C (PKC) and subsequently to phosphorylation of the Na^+-H^+ exchanger, Na^+ accumulation and intracellular alkalosis⁸. The resulting stretch-induced rise in $[Ca^{2+}]_i$ might be

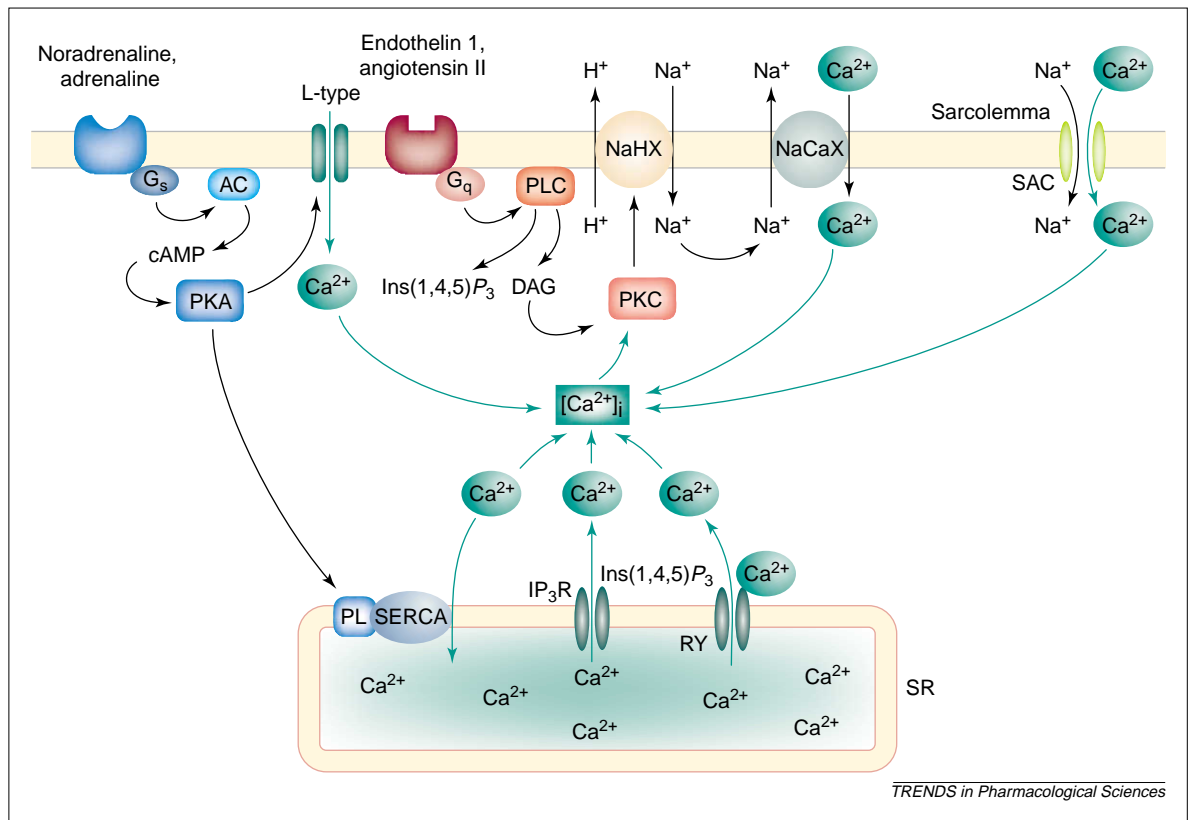
caused by increased Na^+-Ca^{2+} -exchanger activity compensating the Na^+ load. Additionally, ET-1-induced inositol (1,4,5)-trisphosphate [$Ins(1,4,5)P_3$] receptor activation can increase the release of Ca^{2+} from the SR (Ref. 9). The magnitude of this effect is, however, dependent on the cardiac tissue type because atrial cells express more $Ins(1,4,5)P_3$ receptors than do ventricular cells⁹. Stretch-induced alkalinization by ET-1 might play a major role in the ischemic heart because intracellular acidosis suppresses the contractile response to stretch¹⁰ in a similar manner as inhibition of the Na^+-H^+ exchanger⁸. In acidotic heart muscle, the stretch response of the contraction is slower and is accompanied by Ca^{2+} accumulation into the cytosol¹⁰.

Decoding the load signal

Because stretch promotes changes in $[Ca^{2+}]_i$, recent advances have focused on enzymatic pathways (e.g. kinases and phosphatases) that could decode (Fig. 1) the load-induced changes in the amplitude or frequency of Ca^{2+} transients. Several potential Ca^{2+} decoders are activated by the intracellular Ca^{2+} -binding protein calmodulin (CaM).

Overexpression of CaM in transgenic mice hearts promotes hypertrophy¹¹, and α -adrenoceptor stimulation-induced hypertrophy can be inhibited by the CaM antagonist W7 (Ref. 12). The CaM- Ca^{2+} complex acts by binding to specific enzymes [e.g. CaM-kinases (Fig. 3)], thereby enabling the decoding of Ca^{2+} signals. The most studied CaM-activated kinase is the multifunctional CaM-kinase II (CaMKII), which regulates several Ca^{2+} -handling proteins and therefore has profound effects on the Ca^{2+} signal itself¹³. Thus, even if the ET-1-induced hypertrophy can be antagonized by inhibiting

Fig. 2. Suggested Ca^{2+} sources during cardiac mechanotransduction. Stretch induces Ca^{2+} and Na^{+} influx by activation of the stretch-activated cationic channels (SAC). The release of autocrine and paracrine factors such as angiotensin II and endothelin-1, triggered by cardiac wall stretch, might result in activation of additional pathways. Stimulation of endothelin ET_A receptors (G_q -protein-coupled receptor) can cause activation of protein kinase C (PKC) via activation of phospholipase C (PLC). Subsequent phosphorylation of the Na^{+} - H^{+} -exchanger (NaHX) leads to a Na^{+} accumulation and alkalosis. The resulting stretch-induced rise in $[\text{Ca}^{2+}]_i$ might be caused by increased Na^{+} - Ca^{2+} -exchanger (NaCaX) activity compensating the Na^{+} load. In addition to these signaling pathways, increased sympathetic stimulation promotes phosphorylation of Ca^{2+} -handling proteins [e.g. L-type Ca^{2+} channels and phospholamban (PL)], via activation of G_s -protein-coupled receptors, resulting in an increase in the systolic Ca^{2+} concentration. In intact heart, β -adrenoceptor stimulation increases the heart rate (by influencing the firing rate of the pacemaker cells), which leads to an increase of the time-integral of cytosolic Ca^{2+} . Abbreviations: AC, adenylyl cyclase; DAG, diacylglycerol; $\text{Ins}(1,4,5)\text{P}_3$, inositol (1,4,5)-trisphosphate; IP_3R , inositol (1,4,5)-trisphosphate receptor; PKA, protein kinase A; RY, ryanodine receptor; SERCA, sarcoplasmic reticulum Ca^{2+} -ATPase; SR, sarcoplasmic reticulum.



CaMKII with KN62 (Ref. 14), it is not known whether the inhibition is due to altered Ca^{2+} signaling or has a more direct effect on the development of stretch-induced hypertrophy. Other CaMK isoforms might also contribute to the hypertrophy, as suggested by recent findings in mice overexpressing CaMKIV (Ref. 15).

Ca^{2+} -CaM also regulates phosphatases, one of which is calcineurin¹⁶ (Fig. 1), which has been proposed to link intracellular Ca^{2+} signaling pathways to the hypertrophic response in the heart (Fig. 3). Indeed, calcineurin is activated during load-induced cardiac hypertrophy^{17,18}, and transgenic mice that express activated forms of calcineurin develop hypertrophy¹⁹. However, contradictory results have been obtained using the calcineurin inhibitors cyclosporin A and FK506 in pressure-overload models of hypertrophy. Some reports demonstrate prevention of cardiac hypertrophy by cyclosporin A or FK506 in response to mechanical overload^{17,19}, whereas others have shown the development of severe hypertrophy in experimental animals in spite of treatment with calcineurin inhibitors^{20–22}.

Load-related phospholipase C (PLC) stimulation (Fig. 3) by, for example, stretch or ET-1 recruits the PKC signaling cascade. Consistent with a role of this cascade in the development of hypertrophy, transgenic mice with a transiently active mutant of the G protein G_q (Ref. 23) or overexpression of $\text{PKC}\beta$ (Ref. 24) rapidly develop cardiac hypertrophy, and overexpression of $\text{PKC}\beta$ leads to compromised cardiac function. By contrast, $\text{PKC}\epsilon$ overexpression leads to a concentric

hypertrophy²⁵ with normal cardiac function. Thus, PKC subtypes might possess different roles in the development of cardiac hypertrophy. PKC activity is linked to components of mitogen-activated protein kinase (MAPK) signaling systems (Fig. 3) that are able to induce a hypertrophic phenotype in cardiac myocytes²⁶. In addition, the ET-1-activated pathway shows synergism with the CaM-dependent signaling, and ET-induced myocyte hypertrophy can be prevented by inhibition of either CaMKII or calcineurin¹⁴. Calcineurin promotes activation in cardiac myocytes of PKC and the c-JUN N-terminal kinase (JNK) in cardiac myocytes²⁷, which are both able to promote the development of hypertrophy. Additionally, the activity of some Ca^{2+} -sensitive isoforms of PKC depends on the frequency of the Ca^{2+} transients²⁸.

Physiological feedback mechanisms decreasing the load signal

An essential part of cardiac mechanotransduction is the activation of neurohormonal mechanisms that increase myocardial contractility and decrease hemodynamic load (Fig. 1). One main feedback mechanism suppressing the load signal is formed by the cardiac hormones atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP), which are synthesized, stored and released from atrial and ventricular tissue in response to increased wall stretch²⁹. These hormones bind to specific receptors located on cardiac, endothelial, kidney tubular and vascular smooth muscle cells, activating guanylate cyclase (GC) (Fig. 3). The resulting production of

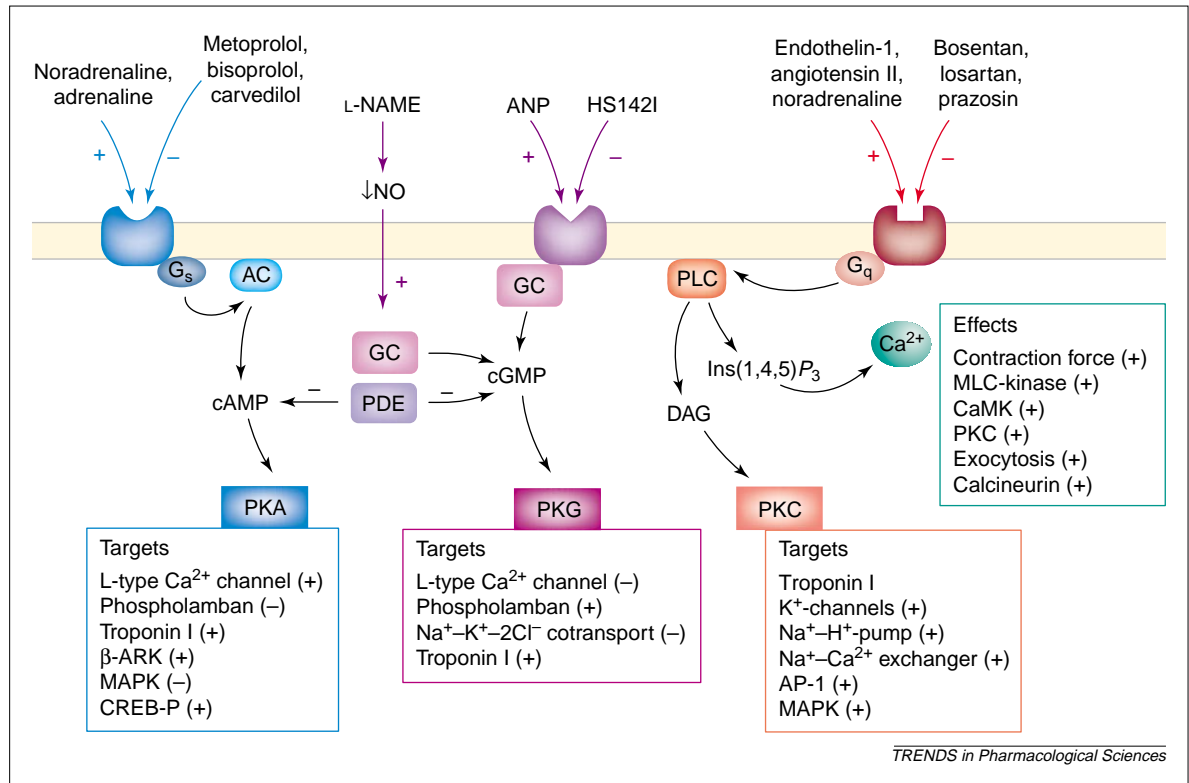


Fig. 3. Neurohumoral interactions and second messenger systems during load-induced cardiac hypertrophy. The enzymatic cascades activated by a variety of hormonal agents can be summarized to involve activation of phospholipase C (PLC), adenylyl cyclase (AC) or guanylate cyclase (GC). Some of the newly developed receptor antagonists with potential clinical value are also shown. G_q -protein-coupled receptors: bosentan is a mixed endothelin ET_A - ET_B receptor antagonist; losartan is an angiotensin AT_1 receptor antagonist; and prazosin is an α_1 -adrenoceptor antagonist. Atrial natriuretic peptide (ANP) GC-coupled receptors: HS1421 (mixture of several esterified linear β -1,6-glucans) is an ANP receptor antagonist. G_s -protein-coupled receptors: metoprolol, bisoprolol and carvedilol are β -adrenoceptor antagonists. Cyclic nucleotide (i.e. cAMP and cGMP) degradation is controlled by phosphodiesterases (PDEs), including several isoenzymes, activated or inhibited by a wide variety of factors (e.g. PDEI is activated by Ca^{2+} -calmodulin, PDEII is activated by cGMP, and PDEIII is inhibited by cGMP). The non-receptor-coupled soluble GC activation is conventionally inhibited by N^G -nitro-L-arginine methyl ester (L-NAME) and similar compounds, via inhibition of nitric oxide (NO) production. Abbreviations: AP-1, activator protein 1; β -ARK, β -adrenoceptor protein kinase; CaMK, Ca^{2+} -calmodulin kinase; CREB-P, cAMP response element-binding protein; DAG, diacylglycerol; MAPK, mitogen-activated protein kinase; MLC, myosin-light chain; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; $\text{Ins}(1,4,5)\text{P}_3$, inositol (1,4,5)-trisphosphate.

cGMP mediates physiological effects such as an increase in glomerular filtration rate, enhanced renal Na^+ excretion, peripheral vasodilatation and attenuation of the activation of the sympathetic and renin-angiotensin systems³⁰.

The feedback mechanisms with positive inotropic effects include increased β -adrenoceptor stimulation, a major regulator of cardiac performance. Contractility of the myocytes is increased by phosphorylation of L-type Ca^{2+} channels and phospholamban (PL) by protein kinase A (PKA) (Fig. 3). Phosphorylation of the Ca^{2+} channels leads to an increase of Ca^{2+} influx during the action potential, whereas PL phosphorylation increases the activity of SR Ca^{2+} -ATPase (SERCA), resulting in faster relaxation (Fig. 2). The contribution of different β -adrenoceptor subtypes to the development of hypertrophy varies. Transgenic mice expressing a 5–15-fold increase in β_1 -adrenoceptor density develop a dilated cardiomyopathy with significant ventricular remodeling³¹. By contrast, mice with robust overexpression of β_2 -adrenoceptors exhibit no signs of cardiomyopathy, despite their enhanced

contractility³¹. Extensive β -adrenoceptor stimulation could lead to hypertrophy by at least two pathways: activation of the PKA-dependent pathway³² and recruitment of the Ca^{2+} -sensitive pathways, which augment both the amplitude (phosphorylation of L-type Ca^{2+} channels and SERCA) and the frequency of Ca^{2+} transients (increased firing rate of the nodal pacemaker cells). A reduced responsiveness to β -adrenoceptor stimulation in the hypertrophied heart is a sum of many factors, such as increased expression of G_i or decreased expression of G_s and adenylyl cyclase (AC), but might, in part, be a consequence of the activation of β -adrenoceptor kinase (β -ARK), an enzyme that 'uncouples' the associated G protein from the activating receptor³¹.

Mechanotransduction in cardiac tissue is often accompanied by expression and release of growth-promoting factors that amplify the growth signal triggered by the mechanical stimulus itself (Fig. 1d). During myocyte hypertrophy, recruitment of these autocrine and paracrine factors might initially be compensatory but as a chronic adjustment they are deleterious. Many studies have suggested an

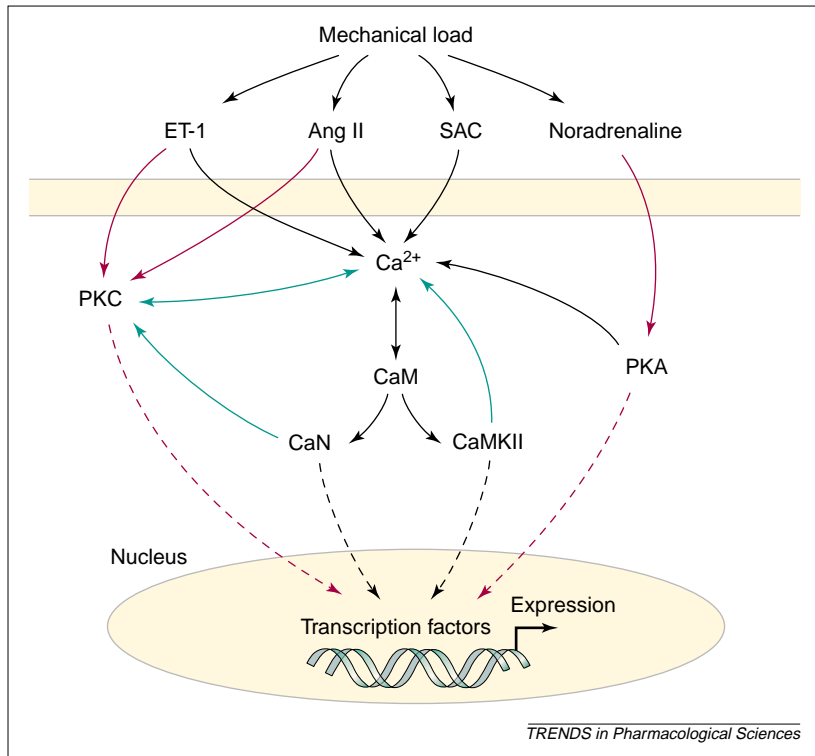


Fig. 4. Crosstalk and synergism of signal cascades activated during cardiac mechanotransduction. Mechanical load-induced production of endocrine and paracrine factors [e.g. endothelin-1 (ET-1), angiotensin II (Ang II) and noradrenaline] and direct effects on stretch-activated channels (SAC) activate Ca^{2+} -dependent pathways (black arrows) and Ca^{2+} -independent pathways (red arrows). Each pathway alone is able to promote changes in gene transcription, acting via the same or different transcription factors. The evaluation of the contribution of each individual pathway in the load-induced changes is a complex task as a result of the interactions of the cascades (green arrows). The broken arrows represent a relationship with possibly more than one step. For a detailed description of the cascades see Refs 15, 19, 26, 32. Abbreviations: CaM, calmodulin; CaN, calcineurin; CaMKII, CaM-kinase II; PKA, protein kinase A; PKC, protein kinase C.

important role for Ang II, via the angiotensin AT_1 receptor, in the development of stretch-induced cardiac hypertrophy^{6,33}. ET-1 might also be involved because it is released rapidly when cultured endothelial cells are stretched³⁴, and its production has been shown to increase in the pressure-overload rat heart³⁵. Furthermore, stretch might trigger the release of Ang II, which in turn releases ET-1 (Ref. 8). However, studies using losartan (an AT_1 receptor antagonist) or bosentan (a mixed ET_A - ET_B receptor antagonist) suggest that Ang II and ET-1 are not essential for wall stretch-induced increases in gene expression in ventricles³⁶. In agreement with this, pressure overload produces marked hypertrophy in transgenic mice with deletion of the AT_{1a} receptor³⁷, showing that Ang II, although clearly a contributing factor, is not essential for load-induced hypertrophy. However, the involvement of these autocrine and paracrine factors in mechanotransduction gives rise to numerous targets for pharmacological intervention (Fig. 3).

From signaling to gene transcription

Transcription factors that regulate gene expression are among the targets of load-activated kinases and phosphatases (Fig. 4). Binding sites for several transcription factors, including transcriptional enhancer factor 1 (TEF-1), activating protein 1 (AP-1) and serum response element (SRE), might be important in mediating the *in vitro* response to hypertrophic signals in neonate cardiocyte cultures³⁸. Whether these findings reflect the *in vivo* situation of the hypertrophied adult heart is not clearly established. The zinc finger transcription factor GATA-4 has been shown to activate not only numerous hypertrophic marker genes, including those encoding BNP, ANP,

β -myosin heavy chain (β -MHC) and TnC (Ref. 39) but also genes expressed during normal adaptation such as that encoding the Na^+ - Ca^{2+} exchanger⁴⁰. Calcineurin dephosphorylates the cytoplasmic transcription factor of activated T cells (NF-AT), leading to entry of NF-AT into the nucleus and cooperative binding to DNA with AP-1, GATA-4 or other transcription factors⁴¹. The recently described CaMKIV pathway activates the myocyte enhancer-binding factor 2 (MEF2) transcription factor¹⁵, acting synergistically with GATA-4 (Ref. 42). Accordingly, it has been suggested that different transcription factors act in synergy to produce the signal-specific expression response⁴³.

Hemodynamic load-induced hypertrophy is accompanied by complex changes in gene programming^{44,45} (Fig. 1). These changes include rapid (within 1 h) and transient upregulation of immediate-early genes that encode nuclear transcription factors (i.e. *c-Fos*, *c-Jun* and *Egr-1*) and BNP (Ref. 46). In the medium term (12–24 h), cardiomyocytes activate the fetal gene regulatory program with re-expression of genes encoding ANP, skeletal muscle α -actin and β -MHC. By contrast, several genes that encode membrane proteins are downregulated in hypertrophied hearts, including SERCA and the β -adrenoceptor. Shifts in gene expression for proteins involved in energy metabolism have also been described. Some of these changes, such as the increased expression of the slow myosin ATPase isoform β -MHC instead of the fast myosin ATPase α -MHC, are adaptive and promote a more favorable energy economy⁴⁵. Normal exercise-induced adaptation of cardiac muscle to an increase of the mechanical load leads to an improvement in heart function and healthy muscle growth, a situation referred to as athlete's heart. The development of pathological hypertrophy leads to opposite changes in function, including improper relaxation and slower contraction kinetics^{45,47} (Fig. 1). This pathological development also compromises mechanotransduction. Trabeculae from human hearts with end-stage dilated cardiomyopathy exhibit length-dependent activation of contraction (Frank–Starling phenomenon), but the length–force curve is flatter than in the normal heart⁴⁸.

The transition from hypertrophic to dilated cardiomyopathy is characterized by dilatation and thinning of the ventricular chambers, which might include the progressive loss of working cardiomyocytes as a result of apoptosis⁴⁵ (Fig. 1). Loss of myocytes in the failing heart would augment the mechanical load on the remaining myocytes. Apoptosis has been shown to be induced by a variety of factors, mostly the same as those that induce hypertrophy, such as mechanical stretch and pressure overload⁴⁹. Recent studies implicate cytokine receptors as possible mediators of stretch-induced apoptosis⁵⁰. Thus, mechanical loading of the myocardium can induce the activation of hypertrophic pathways, but also those of myocyte

survival and cell death. The development of pharmacological agents that stimulate the survival pathway might provide ways to prevent the transition from compensatory hypertrophy to heart failure.

Current treatment strategy

The management of heart failure with left ventricular hypertrophy and systolic dysfunction includes the combined use of angiotensin converting enzyme (ACE) inhibitors, β -adrenoceptor blockers, diuretics and digoxin. This strategy improves the prognosis in heart failure with ACE inhibitors^{51,52} and β -adrenoceptor blockers^{53,54}, and spironolactone in advanced heart failure⁵⁵. Despite recent developments in the pharmacological treatment of hypertension, the common cause of hypertrophy, the regression of left ventricular hypertrophy tends to remain incomplete. In a meta-analysis of 109 trials involving over 2300 hypertensive patients⁵⁶, the left ventricular mass was reduced by 11%, which is far less than the extent of regression of left ventricular mass observed, for example, after valve replacement (35% reduction)⁵⁷.

The clinical relevance of the load-reducing strategy has been shown recently by using the natriuretic peptides for monitoring the mechanical load of the heart⁵⁸. Plasma natriuretic peptide concentrations are independent markers of cardiac status and prognosis in cardiac disease including heart failure⁵⁹. To date, BNP, and particularly its aminoterminal portion (N-BNP), appear to be the most powerful neurohumoral predictors of left-ventricular function and prognosis⁶⁰. Troughton *et al.*⁵⁸ guided the pharmacotherapy of heart failure (e.g. ACE inhibitors, other vasodilators and diuretics) according to the plasma concentrations of N-BNP, and showed that circulating N-BNP concentrations, as an indicator of the load of the left ventricle, could be reduced by intensification of drug therapy⁵⁸. Furthermore, N-BNP-guided treatment of heart failure reduced the total number of cardiovascular events and delayed the time to first event compared with intensive clinically guided treatment. Although the intense suppression of the renin-angiotensin system as well as activation of the bradykinin system by ACE inhibitors might also produce beneficial effects unrelated to load⁶¹, the studies by Troughton *et al.*⁵⁸ are important in the development of strategies for therapy beyond the search for specific drugs.

Future perspectives

To date, it has been demonstrated that myocardial stretch rapidly activates a plethora of intracellular

signaling pathways (Figs 1–3). When seeking the signal cascade from stretch to altered gene expression, myocyte hypertrophy and heart failure, simple mechanisms, with one event leading to another in a precise manner, are not likely to be found. It is more probable that the development of the hypertrophy includes synergism of signal pathways (Fig. 4). Increased understanding of the cellular components of mechanotransduction and knowledge about their interactions are necessary for a successful strategy, but also raises the possibility of inhibiting the development of hypertrophy by specific inhibition of stretch-activated ionic channels, kinases, phosphatases or transcription factors. Recent cloning of the eukaryotic SA-channels^{62,63} provides a possible target for drug development but the usefulness of any SA-channel blockers in therapy or prevention of hypertrophy is unknown. Furthermore, as long as the specific mechanisms that activate the reprogramming of cardiac gene expression are not known, inhibition of any of the known signaling molecules might have both favorable and undesirable effects. As an example of the latter, inhibition of calcineurin has been shown to prevent the development of favorable exercise-induced left ventricular hypertrophy⁶⁴.

The enhancement of the negative feedback pathways in mechanotransduction could be used for therapy, regardless of the specific pathways triggered in the cardiac tissue. One example of this are the new antihypertensive drugs such as vasopeptidase inhibitors (VPIs)⁶⁵. VPIs are dual action molecules that produce simultaneous inhibition of both ACE and neutral endopeptidase (NEP)⁶⁶, which is the principal enzyme involved in the degradation of natriuretic peptides, adrenomedullin and bradykinin. Because these inhibitors change the balance between endogenous vasoconstrictor (i.e. Ang II) and vasodilators (i.e. natriuretic peptides, adrenomedullin and bradykinin) more effectively than does ACE inhibition alone, they could be more useful in the treatment of patients with hypertension and heart failure. Human studies using omapatrilat, the most clinically advanced VPI, have demonstrated a powerful dose-dependent reduction of systolic and diastolic blood pressures⁶⁵. Furthermore, in a double-blind randomized clinical trial in heart failure, omapatrilat showed some advantages over lisinopril in the treatment of patients with congestive heart failure⁶⁷.

Acknowledgements

We thank Andrew French and Olli Vuolteenaho for valuable comments on the manuscript. This work was supported by Academy of Finland, Sigrid Juselius Foundation, Wihuri Foundation, Finnish Foundation for Cardiovascular Research and Paavo Nurmi Foundation.

References

- 1 Tavi, P. *et al.* (1998) Mechanisms of stretch-induced changes in $[Ca^{2+}]_i$ in rat atrial myocytes: role of increased troponin C affinity and stretch-activated ion channels. *Circ. Res.* 83, 1165–1177
- 2 Zhang, Y.H. *et al.* (2000) Stretch-activated and background non-selective cation channels in rat atrial myocytes. *J. Physiol.* 523, 607–619
- 3 Kentish, J.C. and Wrzosek, A. (1998) Changes in force and cytosolic Ca^{2+} concentration after length changes in isolated rat ventricular trabeculae. *J. Physiol.* 506, 431–444
- 4 Wu, Z. *et al.* (1999) Regulation of stretch-activated intracellular calcium transients by actin filaments. *Biochem. Biophys. Res. Commun.* 261, 419–425
- 5 Levijoki, J. *et al.* (2000) Further evidence for the cardiac troponin C mediated calcium sensitization by levosimendan: structure–response and binding analysis with analogs of levosimendan. *J. Mol. Cell. Cardiol.* 32, 479–491
- 6 Dostal, D.E. and Baker, K.M. (1999) The cardiac renin-angiotensin system. Conceptual, or a regulator of cardiac function. *Circ. Res.* 85, 643–650
- 7 Russell, F.D. and Molenaar, P. (2000) The human heart endothelin system: ET-1 synthesis, storage, release and effect. *Trends Pharmacol. Sci.* 21, 353–359
- 8 Alvarez, B.V. *et al.* (1999) Mechanisms underlying the increase in force and Ca^{2+} transient that follow stretch of cardiac muscle: a possible explanation of the Anrep effect. *Circ. Res.* 85, 716–722

- 9 Lipp, P. *et al.* (2000) Functional InsP_3 receptors that may modulate excitation-contraction coupling in the heart. *Curr. Biol.* 10, 939–941
- 10 Tavi, P. *et al.* (1999) Intracellular acidosis modulates the stretch-induced changes in E–C coupling of the rat atrium. *Acta Physiol. Scand.* 167, 203–213
- 11 Gruver, C.L. *et al.* (1993) Targeted developmental overexpression of calmodulin induces proliferative and hypertrophic growth of cardiomyocytes in transgenic mice. *Endocrinology* 133, 376–388
- 12 Sei, C.A. *et al.* (1991) The α -adrenergic stimulation of atrial natriuretic factor expression in cardiac myocytes requires calcium influx, protein kinase C, and calmodulin-regulated pathways. *J. Biol. Chem.* 266, 15910–15916
- 13 Li, L. *et al.* (1997) The effect of Ca^{2+} -calmodulin-dependent protein kinase II on cardiac excitation-contraction coupling in ferret ventricular myocytes. *J. Physiol.* 501, 17–31
- 14 Zhu, W. *et al.* (2000) Ca^{2+} /calmodulin-dependent kinase II and calcineurin play critical roles in endothelin-1-induced cardiomyocyte hypertrophy. *J. Biol. Chem.* 275, 15239–15245
- 15 Passier, R. *et al.* (2000) CaM kinase signaling induces cardiac hypertrophy and activates the MEF2 transcription factor *in vivo*. *J. Clin. Invest.* 105, 1395–1406
- 16 Crabtree, G.R. (1999) Generic signals and specific outcomes: signaling through Ca^{2+} , calcineurin, and NF-AT. *Cell* 96, 611–614
- 17 Lim, H. *et al.* (2000) Calcineurin expression, activation, and function in cardiac pressure-overload hypertrophy. *Circulation* 101, 2431–2437
- 18 Shimoyama, M. *et al.* (1999) Calcineurin plays a critical role in pressure overload-induced cardiac hypertrophy. *Circulation* 100, 2449–2454
- 19 Molkenin, J.D. *et al.* (1998) A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 93, 215–228
- 20 Ding, B. *et al.* (1999) Pressure overload induces severe hypertrophy in mice treated with cyclosporine, an inhibitor of calcineurin. *Circ. Res.* 84, 729–734
- 21 Luo, Z. *et al.* (1998) Calcineurin inhibitors and cardiac hypertrophy. *Nat. Med.* 4, 1092–1093
- 22 Zhang, W. *et al.* (1999) Failure of calcineurin inhibitors to prevent pressure-overload left ventricular hypertrophy in rats. *Circ. Res.* 84, 722–728
- 23 Mende, U. *et al.* (1999) Signal transduction in atria and ventricles of mice with transient cardiac expression of activated G protein α_q . *Circ. Res.* 85, 1085–1091
- 24 Bowman, J.C. *et al.* (1997) Expression of protein kinase C β in the heart causes hypertrophy in adult mice and sudden death in neonates. *J. Clin. Invest.* 100, 2189–2195
- 25 Takeishi, Y. *et al.* (2000) Transgenic overexpression of constitutively active protein kinase C ϵ causes concentric cardiac hypertrophy. *Circulation* 102, 1218–1223
- 26 Sudgen, P.H. (1999) Signaling in myocardial hypertrophy. Life after calcineurin? *Circ. Res.* 84, 633–646
- 27 De Windt, L.J. *et al.* (2000) Calcineurin promotes protein kinase C and c-jun NH_2 -terminal kinase activation in the heart. *J. Biol. Chem.* 275, 13571–13579
- 28 Oancea, E. and Meyer, T. (1998) Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. *Cell* 95, 307–318
- 29 Ruskoaho, H. (1992) Atrial natriuretic peptide: synthesis, release, and metabolism. *Pharmacol. Rev.* 44, 479–602
- 30 Levin, E.R. *et al.* (1998) Natriuretic peptides. *New Engl. J. Med.* 339, 321–328
- 31 Koch, W.J. (2000) Functional consequences of altering myocardial adrenergic receptor signaling. *Annu. Rev. Physiol.* 62, 237–260
- 32 Sudgen, P.H. and Bogoyevitch, M.A. (1995) Intracellular signalling through protein kinases in the heart. *Cardiovasc. Res.* 30, 478–492
- 33 Malhotra, R. *et al.* (1999) Mechanical stretch and angiotensin II differentially upregulate the renin-angiotensin system in cardiac myocytes *in vitro*. *Circ. Res.* 85, 137–146
- 34 McClellan, G. *et al.* (1994) Endothelial cell storage and release of endothelin as a cardioregulatory mechanism. *Circ. Res.* 75, 85–96
- 35 Kaddoura, S. *et al.* (1996) Endothelin-1 is involved in norepinephrine-induced ventricular hypertrophy *in vivo*. Acute effects of bosentan, an orally active, mixed endothelin ET_A and ET_B receptor antagonist. *Circulation* 93, 2068–2079
- 36 Magga, J. *et al.* (1997) Endothelin-1 is involved in stretch-induced early activation of B-type natriuretic peptide gene expression in atrial but not in ventricular myocytes. *Circulation* 96, 3053–3062
- 37 Harada, K. *et al.* (1998) Pressure overload induces cardiac hypertrophy in angiotensin II type 1A receptor knockout mice. *Circulation* 97, 1952–1959
- 38 Sadoshima, J. and Izumo, S. (1997) The cellular and molecular response of cardiac myocytes to mechanical stress. *Annu. Rev. Physiol.* 59, 551–571
- 39 Charron, F. and Nemer, M. (1999) GATA transcription factors and cardiac development. *Semin. Cell Dev. Biol.* 10, 85–91
- 40 Nicholas, S.B. and Philipson, K.D. (1999) Cardiac expression of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger NCX1 is GATA factor dependent. *Am. J. Physiol.* 277, H324–H330
- 41 Rao, A. *et al.* (1997) Transcription factors of the NFAT family: regulation and function. *Annu. Rev. Immunol.* 15, 707–747
- 42 Morin, S. *et al.* (2000) GATA-dependent recruitment of MEF2 proteins to target promoters. *EMBO J.* 19, 2046–2055
- 43 Durocher, D. and Nemer, M. (1998) Combinatorial interactions regulating cardiac transcription. *Dev. Genet.* 22, 250–262
- 44 Chien, K.R. *et al.* (1993) Transcriptional regulation during cardiac growth and development. *Annu. Rev. Physiol.* 55, 77–95
- 45 Swynghedauw, B. (1999) Molecular mechanisms of myocardial remodeling. *Physiol. Rev.* 79, 215–262
- 46 Magga, J. *et al.* (1994) Brain natriuretic peptide in plasma, atria, and ventricles of vasopressin- and phenylephrine-infused conscious rats. *Endocrinology* 134, 2505–2515
- 47 Houser, R.H. *et al.* (2000) Abnormalities of calcium cycling in the hypertrophied and failing heart. *J. Mol. Cell. Cardiol.* 32, 1595–1607
- 48 Vahl, C.F. *et al.* (1998) Length dependence of calcium- and force-transients in normal and failing human myocardium. *J. Mol. Cell. Cardiol.* 30, 957–966
- 49 Hunter, J.J. and Chien, K.R. (1999) Signaling pathways for cardiac hypertrophy and failure. *New Engl. J. Med.* 341, 1276–1283
- 50 Hirota, H. *et al.* (1999) Loss of a gp130 cardiac muscle cell survival pathway is a critical event in the onset of heart failure during biomechanical stress. *Cell* 97, 189–198
- 51 The CONSENSUS Trial Study Group (1987) Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). *New Engl. J. Med.* 316, 1429–1435
- 52 The SOLVD Investigators (1991) Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *New Engl. J. Med.* 325, 293–302
- 53 Packer, M. *et al.* (1996) The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. US Carvedilol Heart Failure Study Group. *New Engl. J. Med.* 334, 1349–1355
- 54 CIBIS Investigators and Committees (1999) The cardiac insufficiency bisoprolol study II (CIBIS II): a randomized trial. *Lancet* 353, 9–13
- 55 Pitt, B. *et al.* (1999) The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *New Engl. J. Med.* 341, 709–717
- 56 Dahlof, B. *et al.* (1992) Reversal of cardiovascular structural changes when treating essential hypertension. The importance of the renin-angiotensin-aldosterone system. *Am. J. Hypertens.* 5, 900–911
- 57 Villari, B. *et al.* (1995) Normalization of diastolic dysfunction in aortic stenosis late after valve replacement. *Circulation* 91, 2353–2358
- 58 Troughton, R. *et al.* (2000) Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. *Lancet* 355, 1126–1130
- 59 Sagnella, G.A. (1998) Measurement and significance of circulating natriuretic peptides in cardiovascular disease. *Clin. Sci.* 95, 519–529
- 60 Richards, A.M. *et al.* (1998) Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: new neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. *Circulation* 97, 1921–1929
- 61 Yusuf, S. *et al.* (2000) Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *New Engl. J. Med.* 342, 145–153
- 62 Walker, R.G. *et al.* (2000) A *Drosophila* mechanosensory transduction channel. *Science* 287, 2229–2234
- 63 Lesage, F. *et al.* (2000) Cloning and expression of human TRAAK, a polyunsaturated fatty acids-activated and mechano-sensitive K^+ channel. *FEBS Lett.* 471, 137–140
- 64 Eto, Y. *et al.* (2000) Calcineurin is activated in rat hearts with physiological left ventricular hypertrophy induced by voluntary exercise training. *Circulation* 101, 2134–2137
- 65 Burnett, J.C., Jr (1999) Vasopeptidase inhibition: a new concept in blood pressure management. *J. Hypertens.* 17, S37–S43
- 66 Roques, B.P. *et al.* (1993) Neutral endopeptidase 24.11: structure, inhibition, and experimental and clinical pharmacology. *Pharmacol. Rev.* 45, 87–146
- 67 Rouleau, J.L. *et al.* (2000) Comparison of vasopeptidase inhibitor, omapatrilat, and lisinopril on exercise tolerance and morbidity in patients with heart failure: IMPRESS randomised trial. *Lancet* 356, 615–620

Chemical names

FK506: $\text{C}_{44}\text{H}_{69}\text{NO}_{12}$

KN62: 1-[N,O-bis-(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine

W7: [N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide]