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Review

Mechanical stress-induced cardiac hypertrophy: mechanisms and signal transduction pathways

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Abstract

Cardiac hypertrophy is a well known response to increased hemodynamic load. Mechanical stress is considered to be the trigger inducing a growth response in the overloaded myocardium. Furthermore, mechanical stress induces the release of growth-promoting factors, such as angiotensin II, endothelin-1, and transforming growth factor-β, which provide a second line of growth induction. In this review, we will focus on the primary effects of mechanical stress: how mechanical stress may be sensed, and which signal transduction pathways may couple mechanical stress to modulation of gene expression, and to increased protein synthesis. Mechanical stress may be coupled to intracellular signals that are responsible for the hypertrophic response via integrins and the cytoskeleton or via sarcolemmal proteins, such as phospholipases, ion channels and ion exchangers. The signal transduction pathways that may be involved belong to two groups: (1) the mitogen-activated protein kinases (MAPK) pathway; and (2) the janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway. The MAPK pathway can be subdivided into the extracellular-regulated kinase (ERK), the c-Jun N-terminal kinase (JNK), and the 38-kDa MAPK (p38 MAPK) pathway. Alternatively, the stress signal may be directly submitted to the nucleus via the cytoskeleton without the involvement of signal transduction pathways. Finally, by promoting an increase in intracellular Ca^{2+} concentration stretch may stimulate the calcium/calmodulin-dependent phosphatase calcineurin, a novel hypertrophic signalling pathway.

Keywords: Hypertrophy; Myocytes; Protein kinases; Signal transduction; Stretch/m–e coupling

1. Introduction

Cardiac hypertrophy is a fundamental process of adaptation to an increased workload due to hemodynamic overload [1,2]. Development of cardiac hypertrophy is initially beneficial since it augments the number of contractile units and reduces ventricular wall stress to normal levels according to the law of Laplace. However, the adaptation has its limits and heart failure may ensue. Furthermore, arrhythmias and ischaemic heart disease may develop which increase the risk of sudden death.

During development of cardiac hypertrophy specific changes have been observed in cardiomyocytes, (1) rapid induction of proto-oncogenes and heat shock protein genes ('immediate-early' genes); (2) quantitative and qualitative changes in gene expression; and (3) increased rate of protein synthesis.

The first response to hemodynamic overload is the induction of proto-oncogenes (such as c-*fos*, c-*jun*, and c-*myc*) and heat shock protein genes (such as *hsp70*),

Abbreviations: Ang II, angiotensin II; ANP, atrial natriuretic peptide; $[Ca^{2+}]_i$, intracellular calcium concentration; DAG, diacylglycerol; ECM, extracellular matrix; ERK, extracellular-regulated kinase; ET-1, endothelin-1; FAC, focal adhesion complex; FAK, focal adhesion kinase; G protein, guanine nucleotide-binding protein; IE genes, immediate-early genes; JAK, Janus-associated kinase; JNKc, Jun N-terminal protein kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MHC, myosin heavy chain; NHE, Na^+/H^+exchanger; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; p38 MAPK, 38 kDa mitogen-activated protein kinase; $p70^{S6K}$, 70 kDa S6 kinase; $p90^{RSK}$, 90 kDa ribosomal S6 kinase (=MAPKAPK1); SAC, stretch-activated channel; SAPK, stress-activated protein kinase; STAT, signal transducers and activators of transcription; TGF-β, transforming growth factor-beta

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therefore called 'immediate-early' (IE) genes. The induction of c-*fos*, c-*myc* and *hsp70* by hemodynamic overload was first reported in rat hearts [3–5]. As a later event, the expression of several genes is modulated either qualitatively or quantitatively. The expression of several genes that encode sarcomeric proteins is switched to expression of fetal isoforms, for example transition from cardiac α-actin to skeletal α-actin, and from the α-form of myosin heavy chain (MHC) to the β-MHC form in rodents [6]. In addition, several shifts in isogene expression of proteins involved in energy metabolism have been described [7,8]. Furthermore, the expression of atrial natriuretic peptide (ANP) that is restricted to the atria shortly after birth, is re-expressed in the ventricles upon hemodynamic overload [5,9]. Besides the qualitative changes in gene expression described above, there are also quantitative changes in constitutive expression of genes, i.e. stimulation of gene expression which contribute to hypertrophy, and down-regulation of genes. Several genes that encode membrane proteins are down-regulated in hypertrophied hearts, for example, the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) gene [10,11].

2. Stimuli inducing cardiac hypertrophy

2.1. Mechanical stress

The primary stimulus for cardiac hypertrophy is mechanical stress or an accompanying increase in neural or humoral factors. However, since cardiac hypertrophy can be induced by hemodynamic overload even after adrenoreceptor blockade (humoral) or sympathectomy (neural) [12], it is likely that mechanical stress itself is the primary factor for cardiac hypertrophy in response to hemodynamic overload. Several ex vivo and in vitro experiments support this view. For example, in isolated hearts, increased cardiac load stimulated protein synthesis [13]. Furthermore, stretching cultured cardiomyocytes stimulated protein synthesis and induced alterations in gene expression without involvement of neural or humoral factors [14–18]. Moreover, in vitro experiments using stretched cardiomyocytes have demonstrated effects that were similar to those in the in vivo heart in response to hemodynamic overload, i.e. increased protein synthesis, expression of IE genes, and re-expression of fetal genes [14,15]. Thus, given these experimental findings, it appears that mechanical stress, such as hemodynamic overload, affects cardiomyocytes by stretch primarily.

2.2. Growth factors and hormones

Growth factors and hormones may be involved indirectly in hemodynamic overload-induced cardiac hypertrophy. The expression and/or release of these factors have been reported in hearts that are hypertrophied due to hemodynamic overload, and in cardiomyocytes that are hypertrophied due to stretch. These factors include endothelin-1 (ET-1) [19–21], angiotensin II (Ang II) [22–24], transforming growth factor-β (TGF-β) [25,26], insulin-like growth factor-1 (IGF-1) [21,26], myotrophin [27], and vascular endothelial growth factor (VEGF) [28,29]. Thus, cardiac myocytes and other cell types, such as cardiac fibroblasts, endothelial cells and vascular smooth muscle cells, may secrete growth promoting factors after a mechanical stress stimulus, which induce hypertrophy of cardiomyocytes in an autocrine/paracrine way.

3. Mechanosensors possibly implicated in cardiac hypertrophy

By which mechanisms is hemodynamic overload coupled to induction of intracellular signals that are responsible for the hypertrophic response? There are several candidate mechanisms that belong to two major groups: (i) integrins and the cytoskeleton, and (ii) sarcolemmal proteins.

3.1. Integrins and the cytoskeleton

Integrins are a family of cell-surface receptors that link the extracellular matrix (ECM) to the cellular cytoskeleton at places called focal adhesion sites [30–32]. Integrins are composed of α and β subunit heterodimers that consist of a large extracellular domain, a transmembrane region, and usually a short cytoplasmic domain. The extracellular domain binds to proteins of the ECM or to counter-receptors on other cells, whereas the cytoplasmic domain forms links with cytoskeletal proteins and, as recently discovered, intracellular signaling molecules such as α-actinin and focal adhesion kinase (FAK) [30,33]. Initially, integrins were considered solely as molecules necessary for adhesive interactions between cells and the ECM, regulating cell adhesion, cell growth, and cell motility. Nowadays, it is believed that integrins can also function as signal transducers, that regulate gene expression and cellular growth, at least in non-cardiac cells [34,35].

Stretch of cardiac fibroblasts caused activation of two signal transduction pathways (the so-called ERK and JNK pathway; see Sections 4.1.1 and 4.1.2, respectively) in an integrin (β_1)-dependent and matrix-specific way, indicating that integrins can act as mechanotransducers in cardiac cells [36]. Furthermore, Ross et al. [37] demonstrated that integrins influence the hypertrophic response in cardiomyocytes. Overexpression of β_1 integrin in the cardiomyocyte was found to increase ANP expression and protein synthesis without affecting DNA synthesis. In addition, Kuppuswamy et al. [38] have shown an association of β_3-integrin and two non-receptor kinases, FAK and c-Src, with the cytoskeleton in hypertrophic cat hearts.

Upon integrin-induced phosphorylation of these kinases, they become activated, may recruit Grb2/Sos and then initiate the Ras/ERK signal transduction pathway [39].

There are two views on the mechanism of integrin-mediated signaling and these two views may be complementary [32]. In the first view, integrins transmit signals by organizing the cytoskeleton (actin filaments) through intermediary molecules including α-actinin, talin, vinculin, paxillin, and tensin, thereby stabilizing cell adhesion and regulating cell shape, morphology, and motility [34]. In accordance with this view Wang et al. [40] showed that: (i) β1 integrin induced focal adhesion formation and supported a force-dependent stiffening response; and that (ii) an increase in the cytoskeletal stiffness required an intact cytoskeleton. Their results suggest that mechanical stress is first received by integrins, and that next interlinked actin microfilaments transduce mechanical stress in concert with microtubules and intermediate filaments. Moreover, Bloom et al. [41] suggested that this mechanism even could modulate gene expression. They showed that intermediate filaments transmit mechanical stress to the chromatin and hypothesized that alterations in the chromatin induce modulation of gene expression. Mechanical stress-induced increases in sarcomere length changed the spatial arrangement of the desmin–laminin filament network that link Z-discs to the chromatin, thereby altering the distribution of chromatin, which may initiate gene transcription [41].

The second view is based on the recently discovered co-localization of signaling molecules in focal adhesion complexes (FACs). In this view integrins are regarded as true receptors capable of inducing biochemical signals within the cell that regulate gene expression and cellular growth. Upon clustering of integrins at focal adhesion sites they recruit the non-receptor kinases FAK and Src, cytoskeletal proteins, and signal-transducing molecules (such as Grb2, Sos, Ras, Raf, PLCγ, ERKs, and SAPKs) forming FACs [32,42–44]. In these FACs signaling proteins and their substrates are brought into close proximity, thereby facilitating signal transduction. In fact, integrins may induce activation of FAK with the help of Src [39], which may lead to activation of the ERK pathway through Grb2-Sos-Ras [43] or through activation of PLCγ [45]. Integrins can also collaborate with growth factor receptors and their substrates to phosphorylate their receptor kinases and to activate ERKs and JNKs upon ligand binding [46,47]. Thus, integrins may integrate a variety of different signaling pathways that are activated by both the ECM and growth factors to establish a well-coordinated response. This mechanism may be important in the hemodynamic overload-induced hypertrophic response that is probably induced not only by mechanical stress itself but also by growth factors released upon mechanical stress.

Furthermore, there is a mechanism proposed by Chicurel et al. [48] in which integrins recruit mRNA and ribosomes to FACs upon cell binding to the ECM and application of mechanical stress thereby relocating these protein synthesis components near the sites of signal reception. This mechanism may serve to increase protein synthesis by post-transcriptional changes before gene expression is changed, or may serve to integrate signals that regulate protein synthesis with those signals that are elicited by integrins, growth-factor receptors and mechanical stresses within the same FAC [40,46–48].

Besides integrins, the ECM proteins ligated to the integrins, such as collagens, laminin, fibronectin, and vitronectin [30], play also a role in signal transduction. MacKennna et al. [36] showed that if the cells were cultured on fibronectin, vitronectin, or laminin, stretch of cardiac fibroblasts activated JNK1 whereas stretch activated ERK2 only if the cells were plated on fibronectin. In addition, ECM proteins, such as laminin and collagens, can influence the myofibrillar and cytoskeletal assembly in cardiomyocytes [49]. Furthermore, it was found that besides integrins, heparan sulfate proteoglycans are involved in formation of focal adhesions and actin stress fibers acting cooperatively with integrins in generating signals in fibroblasts plated on fibronectin [50].

3.2. Sarcolemmal proteins: enzymes, ion channels and antiporters

Mechanical stress causes deformation of the sarcolemma which may (in)directly cause conformational changes in proteins (and subsequently activation of them) that are anchored to the inner surface of the cell membranes, or in transmembrane proteins. Examples of sarcolemmal proteins that might be affected by mechanical stress are several effector enzymes such as phospholipases and protein kinase C isoenzymes, ion channels such as the stretch-activated channel, or ion exchangers such as the Na^+/H^+ exchanger.

3.2.1. Phospholipase C and D (PLC and PLD)

Phospholipases are enzymes that catalyse the breakdown of plasma membrane phospholipids thereby generating second messenger molecules. Major families of phospholipase C (PLC) include a protein kinase-regulated PLCγ family, a PLCδ family, and a G protein-regulated PLCβ family [51]. These families are all membrane-coupled and their catalytic activity is dependent on Ca^{2+} [51]. A cytosolic PLC has also been reported (PLC_{cyt}) that is regulated by the βγ subunit of a G protein [52]. The family of phospholipase D (PLD) very likely consists of multiple isoforms, however until now only two have been cloned: PLD1 and PLD2. PLD activation is regulated by small G proteins and PKC and probably by tyrosine kinases [53,54].

Activated PLC can hydrolyze phosphatidylinositol-bisphosphate (PIP_2) into inositol-triphosphate (IP_3) and diacylglycerol (DAG). DAG is a second messenger that causes translocation of PKC isoenzymes from the cytosol to a membrane fraction, thereby activating them [55].

Activated PKC may then reduce the action of PLC and stimulate that of PLD [56]. Activated PLD preferentially hydrolyzes phosphatidylcholine into phosphatidic acid (PtdOH) and choline. PtdOH is converted into DAG by the enzyme PtdOH hydrolase. Through the 'cross-talk' mechanism between PLC and PLD the cell may be supplied with DAG for a prolonged period of time, thereby providing a sustained response [56,57].

There is some evidence that activation of PLC or/and PLD may play a role in mechanical stress-induced hypertrophy of cardiomyocytes [58–60].

3.2.2. Protein kinase C (PKC)

Protein kinase C (PKC) is a serine/threonine protein kinase. The PKC family comprises several isoenzymes that differ in distribution, regulation, and enzymatic activity. The isoenzymes have been categorized into three subclasses: (i) conventional or classical PKCs (cPKCs: α, β, and γ) which are regulated by DAG, phosphatidylserine, and Ca^{2+}; (ii) novel PKCs (nPKCs: δ, ϵ, η, θ, and μ) which are regulated by DAG and phosphatidylserine, but not by Ca^{2+}; and (iii) atypical PKCs (aPKCs: ζ and λ) whose regulation has to be defined although DAG and Ca^{2+} appear not to be involved [61,62]. The function of PKC is regulated by two mechanisms: (i) by phosphorylation that renders it catalytically competent and causes its release into the cytosol; and (ii) by second messengers (DAG, phosphatidylserine) that promote association of PKC with the membrane and activation by release of the pseudosubstrate [55,61].

Downstream signaling from activated PKC involves two main pathways: (i) indirect regulation of nuclear events; and (ii) direct regulation of nuclear events [63]. PKC can phosphorylate Raf directly or indirectly via Ras [64–66], thereby activating Raf and initiating the ERK pathway which results in activation and nuclear translocation of ERK [67]. Furthermore, PKC can phosphorylate proteins, such as IκB, that function as a cytoplasmic anchor for proteins that have nuclear functions such as the transcription factor NFκB (reviewed in [63]). Upon phosphorylation of IκB, NFκB is released and subsequently translocated to the cell nucleus where it exert its function. On the other hand, evidence for a direct nuclear function of PKC is accumulating (reviewed in [63]). In cardiomyocytes, several PKC isoenzymes have been found to translocate from the cytosol to the nuclear envelope after stimulation with phorbol ester [68]. In addition to the two pathways described above, PKC may modify $[Ca^{2+}]_i$ via Raf and MEK (components of the ERK pathway, see Section 4.1.1), in part by regulating the expression of SERCA, the calcium pump of the SR [69].

Activation of PKC in cardiomyocytes has been found to stimulate expression of c-*fos* and skeletal α-actin genes [70] and to activate transcription of β-MHC, MLC-2a, and ANP [71,72] indicating that activation of PKC can induce hypertrophy. Moreover, stretch of cultured cardiomyocytes induced IE gene expression and stimulated protein synthesis, both being suppressed by down-regulation of PKC [73].

3.2.3. Stretch-activated channels (SACs)

Activation of mechanosensitive ion channels has been proposed as the transduction mechanism between mechanical stress and cardiac hypertrophy (reviewed in Ref. [74]). These stretch-activated channels (SACs) allow passage of ions like Na^+, K^+ and Ca^{2+} [75].

Direct Ca^{2+} influx through SACs was reported in cultured chick heart cells that were stimulated by prodding with a pipette [76]. In addition, stretch of cultured cardiomyocytes increased $[Ca^{2+}]_i$ levels most probably via activation of SACs, since this increase was blocked by pre-incubation of the SAC blockers streptomycin and gadolinium ions [77–79]. However, the involvement of SACs in transduction of the mechanical stress stimulus into the nucleus is still controversial. Several studies could not confirm that gadolinium inhibits stretch-induced expression of IE genes and protein synthesis [80–82].

There are several putative mechanisms by which $[Ca^{2+}]_i$ may contribute to the development of cardiac hypertrophy. Increased $[Ca^{2+}]_i$ may enhance PKC activity followed by direct or indirect alterations in gene expression (see above). $[Ca^{2+}]_i$ can also regulate IE gene expression, such as c-*fos*. Elevated $[Ca^{2+}]_i$ activates the calcium/calmodulin-dependent protein kinase, which can phosphorylate and activate cAMP response element-binding protein (CREB), a transcription factor. Upon binding to the calcium response element within the cAMP response element, CREB can induce transcription of c-*fos* (reviewed in Ref. [83]). In addition, $[Ca^{2+}]_i$ regulates expression of several genes by affecting initiation of transcription, mRNA stability and the translation of mRNA into protein [83]. Furthermore, Ca^{2+} ions may also stimulate protein synthesis since it was shown that depletion of intracellular calcium stores inhibited protein synthesis (reviewed in Ref. [84]). Another putative mechanism by which Ca^{2+} ions may regulate hypertrophy involves the Ca^{2+}/calmodulin-dependent protein phosphatase, calcineurin (see Section 4.3).

3.2.4. The Na^+/H^+ exchanger (NHE)

The Na^+/H^+ exchanger (NHE) may also play a role in mechanotransduction since its activation increases intracellular pH (cytoplasmic alkalization) which is known to stimulate expression of hypertrophic marker genes and protein synthesis [85]. NHE is located in the sarcolemma and regulates Na^+ influx and H^+ efflux with a stoichiometry of one to one [86,87].

Using cultured cardiomyocytes, Yamazaki et al. [82] showed that HOE 694, a specific inhibitor of NHE, markedly attenuated stretch-induced activation of the ERK pathway and stimulation of protein synthesis. Furthermore, stretch-induced activation of the MAPK pathway was partially blocked by pretreatment with NH_4Cl (intracellu-

lar acidification), suggesting that cytoplasmic alkalization may be a crucial step to activate the ERK pathway in stretched cardiomyocytes. Autocrinely released Ang II or ET-1 was not related to the stretch-induced NHE activation. On the other hand, Cingolani et al. [88] found in papillary feline muscle that stretch induced a rise in pH_i which was completely blocked by specific inhibition of the NHE, by blockade of the Ang II type 1 receptor and the ET-1 type B receptor, and by inhibition of PKC. These authors concluded that stretch increases pH_i due to enhanced NHE activity which was mediated by PKC, and by autocrine/paracrine release of Ang II and/or ET-1. Thus, the NHE may play a role in converting mechanical stress into a biochemical signal although the influence of autocrine/paracrine factors awaits clarification.

3.2.5. Guanine nucleotide-binding proteins (G proteins)

Another candidate mechanism of mechanotransduction involves guanine nucleotide-binding proteins (G proteins) that couple cell surface receptors to the appropriate effectors. There are two forms of signal transducing G proteins: the 'small G proteins' and the 'heterotrimeric G proteins'. These G proteins share a common characteristic: 'they exist in two interconvertible conformational states, i.e. an inactive guanosine diphosphate (GDP)-bound state and an active guanosine triphosphate (GTP)-bound state' (from Ref. [89]).

The small G proteins are single polypeptides composed of about 200 amino acids, such as the Ras family and Rho family. The Rho family appears to play a role in controlling the organization of the actin cytoskeleton, and in the formation of FACs, i.e. it regulates integrin clustering [90,91]. In addition, two members of the Rho family, namely cdc42 and Rac-1, have been shown to stimulate two distinct MAP kinase families, the JNKs and p38 MAPKs [92,93]. The Ras family is a well-known regulator of the ERK pathway, but mediates several other effector pathways as well, including the JNK pathway [94] (reviewed in Ref. [95]).

The heterotrimeric G proteins are associated with signal transduction originating from cell surface receptors. Heterotrimeric G protein subunits have been shown to be localized at sites of focal adhesions that provide contact via integrins with the ECM thereby functioning as a sensor of mechanical stress [96]. Stretch of cultured neonatal cardiac fibroblasts was found to stimulate G protein activation within 1 min of stretching, the response being modulated by the rate and the magnitude of mechanical stress. Furthermore, immunoprecipitation revealed that $G\alpha_q$ and $G\alpha_{i1}$ were the subunits that become rapidly activated upon mechanical stress [97]. Akhter et al. [98] reported an attenuation of pressure overload-induced hypertrophy in transgenic mice that expressed an inhibitor peptide of the $G\alpha_q$ subunit. In addition, D'Angelo et al. [99] showed induction of marker genes of cardiac hypertrophy, increased heart weight in relation to body weight, and increased cardiomyocyte size in transgenic mice that overexpressed $G\alpha_q$ in a cardiac-specific manner. Activation of PKC appeared to be crucial in this G protein-induced hypertrophy. These studies demonstrated a vital role for heterotrimeric G proteins in mechanotransduction of mechanical stress and cardiac hypertrophy. Activation of heterotrimeric G proteins is a major activation mechanism of PLC, that subsequently can activate PKC [100]. Therefore it is an interesting hypothesis that integrins, heterotrimeric G proteins, PLC and PKC have an integrated action in mechanotransduction. Recently, evidence has been presented in favour of this hypothesis [101,102].

4. Signal transduction of the stretch stimulus

There are two main signal transduction pathways that may be involved in mechanical-stress induced hypertrophic response: (i) the mitogen-activated protein kinase (MAPK) pathway; and (ii) the Janus-associated kinases/signal transducers and activators of transcription (JAK/STAT) (Fig. 1). Nowadays, it is known that there are several interactions between these pathways. In fact, mechanical stress appears to activate both pathways. Recently, a novel

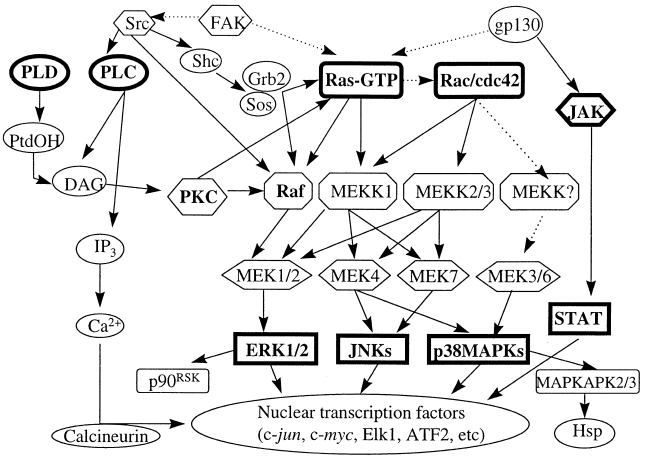

Fig. 1. Signal transduction pathways possibly involved in mechanical stress-induced hypertrophy. These include two major pathways: the MAPK pathway and the JAK/STAT pathway. The MAKP pathway is a three-module cascade of phosphorylating kinases: MEKK→MEK→MAPK. This pathway consists of several subfamilies among which the ERK pathway, the JNK pathway and the p38 MAPK pathway. They are activated by heterotrimeric G proteins coupled to membrane receptors, by small G proteins (such as Ras, cdc42, Rac), by protein kinases (such as Src and FAK), by PKC via activation of PLC or/and PLD, or by JAKs via gp130. Their downstream targets are cytosolic kinases (such as $p90^{RSK}$, and MAPKAPK2/3) and nuclear transcription factors (such as c-jun, c-myc, and Elk1). The JAK/STAT pathway is directly activated probably via gp130. Upon activation of STATs by JAKs, STATs translocate to the nucleus and induce gene transcription. In addition, a direct pathway linking mechanical stress to gene expression has been considered to be operative via the cytoskeleton. The involvement of calcineurin in the development of hypertrophy is still controversial. The dashed lines refer to poorly understood mechanisms.

hypertrophic signaling pathway has been described which involves activation of the Ca^{2+}/calmodulin-dependent phosphatase calcineurin [103]. These three pathways (Sections 4.1–4.3) control gene transcription and may therefore be involved in stretch-induced modulation of gene expression. The rate of protein synthesis is probably increased by a different mechanism (Section 4.4) although its involvement in the development of cardiac hypertrophy has to be proven.

4.1. The mitogen-activated protein kinase (MAPK) pathway

Mitogen-activated protein kinases (MAPKs) are serine/threonine kinases that become activated upon tyrosine/threonine phosphorylation and additional modifications, and then in turn phosphorylate and activate nuclear substrates (such as c-*myc*, c-*jun*, ATF-2, and $p62^{TCF}$) and other kinases (such as $p90^{RSK}$ and MAPKAP kinase 2 [104,105]) (reviewed in Refs. [106,107]). The MAPKs are the final components of the MAPK pathway that consist of a three kinase modules [108–110]: (1) the MAP kinases (MAPKs), (2) the MAPK/ERK kinases (MEKs), and (3) the MEK kinases (MEKKs). The MEKKs are serine/threonine kinases that activate MEKs by dual phosphorylation on a serine and serine/threonine residue lying within a Ser-xxx-Ser/Thr motif [111]. MEKs activate MAPKs by dual phosphorylation on a tyrosine and a threonine residue lying within a Thr-xxx-Tyr motif, i.e. the phosphorylation motif [112]. MAPKs preferentially phosphorylate substrates on Ser/Thr-Pro, although the optimal sequence is Pro-xxx-Ser/Thr-Pro [106]. Thus, the MAPK pathway involves a cascade of phosphorylation of kinases in the following order: MEKK→MEK→MAPK.

The MAPK superfamily is a widely distributed group of enzymes that can be divided in several subfamilies. The three best characterized MAPK cascades are: (i) the extracellular-regulated kinases (ERKs); (ii) the c-Jun N-terminal kinases (JNKs); and (iii) the p38 MAPKs cascade, the latter two belong to the group of stress-activated protein kinases (SAPKs) [108,109,113,114]. The MAPK subfamilies all have different amino acids in their phosphorylation motif which helps to identify them (the above mentioned xxx is Glu for ERKs, Pro for JNKs, and Gly for p38 MAPKs) [108]. Furthermore, at the level of MEK the MAPK pathways may converge [115,116].

4.1.1. The extracellular-regulated kinase (ERK) pathway

There are several extracellular-regulated kinases (ERKs): ERK1-6. The best characterized are: the 44-kDa MAPK (ERK1), the 42-kDa MAPK (ERK2), and the 63-kDa MAPK (ERK3) [117]. In the heart, ERK1 is the most highly expressed ERK. The expressions of three ERK subtypes (ERK1-3) decrease upon maturation; the expression of ERK3 is hardly detectable in adult heart [117]. The MEKs in this pathway are MEK1 and MEK2; the MEKKs are Raf kinase [118,119] and also MEKK1 [110]. Substrates for ERKs are transcription factors, such as c-*jun*, and $p62^{TCF}$ (Elk-1), and the 90-kDa S6 kinase ($p90^{RSK}$) [104,106,107].

Raf with the cofactor 14-3-3 bound to it, is recruited to the membrane by Ras-GTP for phosphorylation by membrane-bound tyrosine kinases [120–122]. Upon phosphorylation of Raf by tyrosine kinases (for example by Src kinases via the linker protein Shc, the adapter protein Grb2, and the guanine nucleotide exchange factor Sos [123]) Raf becomes activated and can initiate the ERK pathway [121]. Alternatively, it has been reported that activation of Raf by Ras can occur without phosphorylation, probably mediated through a conformational change [124]. The activation of ERK2 by Raf however, seems to be Ras-independent [118]. This activation of Raf may involve another mechanism, i.e. via PLC and PKC (see Section 3.2.1), which is independent of tyrosine kinases and Ras [64,125]. Finally, there is a signal transduction pathway leading to ERK activation which involves protein kinase A (PKA) and may be Ca^{2+}-dependent [126,127]. The signal transduction pathways leading to ERK activation may differ among cell types (reviewed in Ref. [128]).

The ERK pathway can be stimulated upon G protein-coupled receptor occupation by binding of hormones (for example by binding of ET-1 [116] and Ang II [129]), and upon occupation of receptors with intrinsic tyrosine kinase activity by binding of growth factors (for example IGF-I [130]) (reviewed in Refs. [108,131,132]). Mechanical stress has also been reported to stimulate this pathway: activation of ERK1 and ERK2, Ras, and $p90^{RSK}$ [59,126,133–138]. The precise mechanism of the stimulation of this pathway is unknown, but appears to involve PKC, tyrosine kinases, and Ras [133,136,137]. Moreover, stretch of cardiomyocytes caused activation of ERKs and resulted in increased expression of c-*fos* and skeletal α-actin, indicating that ERKs and mechanical stress-induced hypertrophy may be linked [59,134]. However, ANP expression may be either not regulated [139] or down-regulated by the ERK cascade [140]. Taken together, these results indicates that the ERKs may partly participate in the mechanisms of mechanical stress-induced hypertrophy.

4.1.2. The c Jun N-terminal protein kinase (JNK) pathway

The members of this pathway and the p38 MAPK pathway were initially identified as stress-activated protein kinases (SAPKs), since they were preferentially activated by environmental stress (reviewed in Ref. [107]). It has now become clear that they belong to two different pathways because of differences in their dual phosphorylation motif, in their upstream activators, and their downstream targets [114]. The c-Jun N-terminal kinases (JNKs) are named after the first substrate identified, the IE gene c-*jun* [141]. The JNKs are encoded by three genes that all produce multiple products by alternative splicing yielding

three isoforms: JNK1 (SAPKγ), JNK2 (SAPKα), and JNK3 (SAPKβ) (terminology after Ref. [114]) [141,142]. All isoforms have an apparent molecular weight of approximately 46 or 54 kDa [114,143]. The JNKs differ in their interaction with transcription factors which provides a tool for selectively targeting of specific transcription factors [143]. The upstream activators of JNKs are not well-defined and poorly studied in heart tissue. MEK4 and MEK7 appear to activate JNKs [107,144]. Furthermore, MEKK1 may be an upstream kinase of the JNK pathway, since it phosphorylates MEK4 which in turn phosphorylates and activates JNKs [145]. MEKK5 is probably also an upstream activator of MEK4 in the JNK pathway, at least in vitro [146]. Also MEKK2 and 3 are able to activate JNKs [110]. Initiation of the JNK pathway may be triggered by Rac and cdc42, members of the Rho family of small G proteins [92,93].

The JNK pathway may play a role in mechanical stress-induced hypertrophy via phosphorylation of the transcription factors c-Jun, and ATF2 [147]. In cardiomyocytes submitted to stretch, JNK activity was maximally increased at about 30 min [148]. This activation of JNKs was independent of secreted Ang II, extracellular Ca^{2+}, and PKC. Others have found that cardiomyocytes submitted to cyclic stretch had a maximal activation of JNKs at about 5 min [133]. Using MEKK1-transfected cardiomyocytes, Thorburn et al. [140] showed that overexpression of MEKK1 induced ANP expression (a marker of hypertrophy). Moreover, MEKK1 stimulates JNKs as well as ERKs. However, the JNK pathway appeared to stimulate ANP expression, whereas the ERK pathway inhibited expression of ANP. Furthermore, these authors found that the small G protein Rho was also required for MEKK1-induced ANP expression [140]. Controversially, Nemoto et al. [149] found that activation of JNKs inhibited MEKK 1-induced ANP expression via a feedback loop of c-*jun*. They reported that ANP expression is activated by p38 MAPKs. So, it seems that the induction of ANP expression is biphasic, first a short-living response upon JNK activation followed by a prolonged response upon p38 MAPK activation.

4.1.3. The p38 MAPK pathway

Another subfamily of the MAPKs is the p38 MAPK family. Until now, four genes have been described encoding six isoforms: p38 MAPKα, p38 MAPKβ, p38 MAPKδ, and p38 MAPKγ [150]). The substrate of p38 MAPK is MAPK-activated protein kinase 2 (MAPKAPK2) [151]. MAPKAPK2 can phosphorylate and thereby activate the small heat shock proteins Hsp 25 (the murine form) and Hsp27 (the human form) [152] that are supposed to be cytoprotective in heart cells [153]. The p38 MAPK cascade also results in phosphorylation of transcription factors, including ATF-2, which regulate gene expression. Upstream activators of the p38 MAPKs are MEK3, MEK6, and probably MEK4 [150,154]. MEKK5 may serve as an upstream kinase of MEK6 [155].

The p38 MAPK pathway may be involved in mechanical stress-induced hypertrophy, since it was found recently that in a mouse model of pressure overload p38 MAPK activity was increased [156]. Furthermore, cyclic stretch of cardiomyocytes not only induced phosphorylation of the ERKs, JNKs, and FAK, but also of p38 MAPK [133]. These results were confirmed by other investigators who reported that stretch of cardiomyocytes derived from angiotensin II type 1a knock-out mice activated ERKs as well as p38 MAPK, followed by induction of c-*fos* expression [138]. To dissect specific functions of the p38 MAPKs, MEK3 and MEK6 were constitutively introduced into cardiomyocytes [150,154]. These experiments suggested that p38 MAPKβ is the subtype that mediates hypertrophy, whereas p38 MAPKα is involved in programmed cell death (apoptosis) [156]. Interestingly, the hypertrophic response induced by p38 MAPKβ included several markers of hypertrophy, i.e. an increase in cell surface area, enhanced organization of sarcomeric proteins, and induction of ANP expression [154,156].

In conclusion, all three subfamilies of MAPKs, i.e. ERKs, JNKs and p38 MAPKs, may play a role in the transduction of mechanical stress into a hypertrophic response. Their precise roles, i.e. induction of protein synthesis, induction of IE gene expression, induction of morphological changes by shifts in isoenzyme expression, and induction of ANP expression, however, await clarification.

4.1.4. The mitogen-activated protein kinase phosphatases (MKPs)

A family of dual-specificity phosphatases, MAP kinase phosphatases (MKPs), can inactivate the MAPK cascades [157,158]. MKPs selectively dephosphorylate phosphothreonine and phosphotyrosine residues leading to inactivation of MAPKs. Until now, there are no studies performed to investigate the role of MKPs in mechanical stress-induced hypertrophy.

4.2. The janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway

Janus-associated kinases (JAKs) were first identified as protein tyrosine kinases associated with cytokine receptors that regulate signal transduction of these receptors [159]. The JAK family consists of Jak1, Jak2, Jak3, and Tyk2. They have a molecular mass ranging from 120 to 130 kDa [159]. Signal transducers and activators of transcription (STATs) are latent transcription factors located in the cytoplasm which become activated by phosphorylation on a tyrosine residue. They are named after their dual functions in signal transduction in the cytoplasm and activation of transcription in the nucleus [160,161]. Several

STAT isoforms have been identified: STAT1, 2, 3, 4, 5a, 5b, and 6 [160,161].

4.2.1. The JAK/STAT cascade

Binding of ligands to their cytokine receptors, such as cardiotrophin-1 (CT-1) [162], leads to phosphorylation and activation of the receptor-JAK complex with subsequent recruitment of STATs and activation of STATs by phosphorylation. The phosphorylated STATs dimerize, migrate into the nucleus, and bind response elements in the promoters of target genes to stimulate gene transcription [159,161]. In case of receptor complexes sharing the glycoprotein 130 (gp130), i.e. members of the interleukin (IL)-6 family such as IL-6 and CT-1, signal transduction is triggered by the formation of dimers of gp130 [163]. Activation of STATs occurs also through receptor families other than the cytokine receptor family such as tyrosine kinase receptors [164] and G protein-coupled receptors (for example, Ang II receptor [165–167]).

The JAK/STAT pathway was found to be activated in rat hearts with pressure overload-induced hypertrophy [168,169]. Activation of the JAK/STAT pathway by overload was mediated by gp130, and at least CT-1 and IL-6 were involved in activation of this pathway [169]. Furthermore, Pan et al. [168] showed that activation of the JAK/STAT pathway by pressure overload contained an Ang II-dependent (Tyk2 and JAK2) and an angiotensin II-independent (JAK1) component. The Ang II-independent component may be represented by a mechanical stress component as illustrated by a later report of these authors [170]. They showed that stretch of cardiomyocytes induced phosphorylation of JAK1, JAK2, Tyk2, and gp130. Furthermore, STAT1 and STAT3 were activated of which the activation of STAT1 was Ang II-dependent. In addition, JAK2 activity was necessary for the stretch-induced STAT1 and STAT3 activation [170]. Stretch-induced secretion of ET-1 appeared not to be involved in phosphorylation of the STATs. Thus, although the participation of the JAK/STAT pathway in development of mechanical stress-induced hypertrophy is poorly studied, the involvement of JAK1, STAT3 and maybe JAK2 seems likely.

The transmembrane glycoprotein gp130 may play a major role in signal transduction since it has been found that gp130 is an upstream activator of the JAK/STAT pathway as well as the ERK pathway [171]. Interestingly, in stretched cardiomyocytes the JAK/STAT pathway is activated [170], and the ERK pathway is activated [133]. Even in cardiomyocytes derived from angiotensinogen-deficient mice that cannot produce Ang II, the ERK pathway is activated by stretch and this effect was regulated by gp130 [172]. Thus, mechanical stress-induced activation of both the ERK pathway and the JAK/STAT pathway may occur via gp130.

4.2.2. Src homology phosphatases (SHPs)

The phosphorylation state and thus the activation state of JAK2 (and possibly other kinases) is controlled by the action of Src homology phosphatase (SHP)-1 [173]. SHP-1 may downregulate the JAK/STAT cascade through dephosphorylation of JAK2 [174,175]. Another phosphatase which regulates protein tyrosine kinase activity is SHP-2 [173]. However, in contrast to SHP-1, SHP-2 probably functions as a positive effector of signal transduction [175,176]. SHP-2 may play a role as an adaptor protein for JAK2 association with G protein coupled receptors, thereby facilitating JAK2 phosphorylation and activation [175].

4.3. Calcineurin-dependent pathway

Recently, Molkentin et al. [103] showed that cardiac hypertrophy can be induced by the Ca^{2+}/calmodulin-dependent phosphatase calcineurin. Transgenic mice that expressed activated forms of calcineurin developed cardiac hypertrophy that could be prevented by cyclosporine, an inhibitor of calcineurin [103]. In addition, cyclosporine also suppressed, besides development of hypertrophy, re-expression of a fetal gene repertoire in cardiomyocytes when stimulated in vitro with Ang II and phenylephrine. Activation of calcineurin dephosphorylates the cytoplasmic transcription factor NF-AT3, that subsequently migrates into the nucleus and interacts with the GATA4 transcription factor to synergistically upregulate gene expression [103]. This calcineurin-dependent pathway may link increases in $[Ca^{2+}]_i$ with induction of cardiac hypertrophy. The involvement of calcineurin in development of cardiac hypertrophy was confirmed by Sussman et al. [177] and Shimoyama et al. [178]. Sussman et al. [177] reported that cyclosporine treatment prevented pressure overload-induced cardiac hypertrophy. Shimoyama et al. [178] reported that a calcineurin inhibitor FK506, inhibited activation of calcineurin and prevented pressure overload-induced cardiac hypertrophy and fibrosis. However, other studies failed to show that cyclosporine suppresses the development of cardiac hypertrophy in rodents with hemodynamic overload in vivo [179,180]. Whether hemodynamic overload activates calcineurin, and whether calcineurin activation plays a crucial role in the development of overload-induced cardiac hypertrophy, remains uncertain at this time.

4.4. Regulation of protein synthesis

The increase in the rate of protein synthesis observed in cardiac hypertrophy may be regulated by phosphorylatable heat- and acid-stable protein I (PHAS-I) in the rat and its human homologue eukaryotic initiation factor 4E binding protein (eI4E-BP) (reviewed in Refs. [108,181]). PHAS-I is involved in initiation of translation of RNA into protein. PHAS-I limits initiation of translation by binding to the eukaryotic initiation factor 4E (eIF4E). Upon phosphorylation of PHAS-I, the PHAS-I/eIF4E complex dissociates, thereby removing the inhibitory effect of PHAS-I on eIF4E and translation is initiated [182]. Phosphorylation of

PHAS-I is mediated by the kinase mammalian target of rapamycin (mTOR) [182], and not by the ERK pathway as was initially assumed [183].

Another mechanism by which the protein synthesis may be stimulated is through activation of the 70-kDa S6 kinase ($p70^{S6K}$). S6 is a component of 40S ribosomal proteins, that regulates initiation and elongation of protein translation [184]. Upon phosphorylation of S6 by S6 kinases, protein synthesis is stimulated. Initially it was assumed that the 90-kDa ribosomal S6 kinase ($p90^{RSK}$) was involved in phosphorylation of S6 [185]. Nowadays it has been suggested that $p70^{S6K}$ is the physiological S6 kinase [186].

5. Interaction between cardiomyocytes and cardiac fibroblasts: autocrine/paracrine mechanisms

The development of cardiac hypertrophy induced by hemodynamic overload is very likely triggered by mechanical stress. However, the involvement of growth promoting factors (such as TGF-β and VEGF), hormones (such as Ang II and ET-I) and cytokines (such as CT-1) cannot be ruled out. We support the view that they are released upon mechanical stress and then act on neighbouring cells. This view is based on a study performed by Sadoshima et al. [59], who found that if stretch-conditioned medium derived from stretched cardiomyocytes is transferred to non-stretched cardiomyocytes, this stretch-conditioned medium induces hypertrophy in the recipient non-stretched cardiomyocytes. Released factors may act on the cells themselves (autocrine mechanism) and on other cell types (paracrine mechanism). The growth promoting factors that were proposed to play a major role in mechanical stress-induced hypertrophy are Ang II, ET-1, and TGF-β.

5.1. Angiotensin II (Ang II)

Angiotensin II (Ang II) is the effector peptide of the renin–angiotensin system (RAS). Nowadays, there is evidence for RAS systems in tissues, such as the myocardium [187]. Not all RAS components are synthesized in the tissue itself, but 'there is a system generating Ang II locally rather than a local RAS' (from Ref. [188]). This locally produced Ang II is known as a factor capable of inducing hypertrophy of cardiomyocytes and hyperplasia of cardiac fibroblasts [189,190].

Mechanical stress of cardiomyocytes induces angiotensinogen expression and promotes Ang II release from secretory granules [22,24,191]. Moreover, upon stretch of cardiomyocytes the increase in c-*fos*, Egr-1, skeletal α-actin and ANP expression, as well as enhanced protein synthesis were suppressed or even completely blocked by an Ang II type 1 (AT_1) receptor blocker [22,24,192]. In addition, the hypertrophic effect of conditioned medium (CM) derived from stretched cardiomyocytes on non-stretched recipient cardiomyocytes was inhibited by addition of an AT_1 receptor blocker to the CM [22,193]. In vivo studies using spontaneously hypertensive rats showed that hypertension-induced cardiac hypertrophy was significantly reduced by treatment with an AT_1 receptor blocker [192]. However, mechanical stress of cardiomyocytes induced ERK activity and stimulated protein synthesis, which were only partially suppressed by an AT_1 receptor blocker [193]. Also, in cardiomyocytes derived from AT_{1A} receptor knockout mice (cardiomyocytes from these rats have no transcripts of AT_{1A} genes, and neither the AT_{1B} nor AT_2 gene are upregulated) mechanical stress still activated ERK activity [138]. Moreover, studies performed in AT_{1A} receptor knockout mice (that have no detectable AT_{1A} mRNA levels, and very low AT_{1B} mRNA levels) showed that pressure overload still induced hypertrophic responses without affecting AT_1 or AT_2 mRNA levels [194,195]. These experiments indicates that 'AT_1-mediated Ang II signaling is not essential for the development of pressure-overload-induced cardiac hypertrophy' [195].

5.2. Endothelin-1 (ET-1)

Endothelin-1 (ET-1) is a vasoconstrictor peptide originally identified from the supernatant of cultured porcine aortic endothelial cells [196]. In cardiomyocytes, ET-1 stimulated hypertrophy as determined by an increase in protein synthesis and cell surface area, expression of IE genes, and induction of ANP, skeletal α-actin, and MLC2a genes [197–199].

Contribution of local ET-1 to hypertrophy of cardiomyocytes was demonstrated by an increase in ventricular ET-1 levels during pressure overload, which showed a positive correlation with the degree of hypertrophy [20]. In situ mRNA hybridization revealed that preproET-1 mRNA was expressed in hypertrophied cardiomyocytes, suggesting that cardiomyocytes can be a source of ET-1 production in hypertrophied hearts [20]. In addition, stretch of cultured cardiomyocytes increased preproET-1 mRNA expression and stimulated the release of ET-1 [19]. A specific ET-1 receptor blocker suppressed the increase in protein synthesis and the activation of Raf and ERK in stretched cardiomyocytes, suggesting a role for ET-1 in mechanical stress-induced hypertrophy [19]. These experiments were confirmed by an in vivo study. In rats submitted to hemodynamic overload, cardiac hypertrophy with concomitant expression of the skeletal α-actin and ANP was partially blocked by the action of an ET-1 type A (ET_A) receptor blocker [200].

5.3. Transforming growth factor-beta (TGF-β)

There are three distinct forms of transforming growth factor-beta (TGF-β), TGF-β_1 [201], TGF-β_2, and TGF-β_3 [202,203]. TGF-β is secreted in a latent form, and proba-

bly becomes activated upon proteolytic cleavage by proteases [204].

The view that TGF-β_1 may play a role in cardiac hypertrophy has derived from two observations: (i) TGF-β_1 induced expression of collagen mRNA followed by deposition of collagen proteins by cardiac fibroblasts; and (ii) TGF-β_1 induced expression of β-MHC and skeletal α-actin in cardiomyocytes [205]. In pressure overloaded hearts, TGF-β_1 mRNA was increased considerably, TGF-β_3 mRNA levels were unchanged, and expression of ECM proteins such as fibronectin and collagen was increased [25,206]. Other investigators showed that this increase in TGF-β_1 mRNA expression upon pressure overload occurred in cardiomyocytes mainly, although basal TGF-β_1 mRNA was localized in fibroblasts predominantly [207]. Upon hypertrophic stimuli such as norepinephrine and stretch, cardiomyocytes secreted increased quantities of TGF-β_1 [207]. Together, these results implicate an autocrine as well as a paracrine role for TGF-β in induction of cardiac hypertrophy, i.e. secretion by cardiomyocytes followed by hypertrophy of cardiomyocytes and increased deposition of ECM proteins by fibroblasts, respectively [208].

6. Conclusions

The identification of integrins, G proteins, and the Na^+/H^+ exchanger as potential mechanosensors, and MAPK and JAK/STAT pathways as potential participants in mechanical stress-induced signal transduction is of great interest. The role of the calcineurin-dependent pathway in mechanical stress-induced hypertrophy is still controversial. Release of growth-promoting factors, such as angiotensin II, endothelin-1, and transforming growth factor-β, upon stretch may stimulate cardiac hypertrophy in an autocrine/paracrine way.

These potential mechanisms that may contribute to overall hypertrophic growth and changed cardiac phenotype have been identified using cell culture data and other models of hypertrophy. Whether these mechanisms apply to pathophysiological hypertrophy induced by mechanical stress in humans is still uncertain. Nevertheless, the data summarized here elucidate the many mechanisms that are involved in development of mechanical stress-induced hypertrophy. It is to be expected that future development of antagonists of specific mechanisms implicated in the development of cardiac hypertrophy will lead to new therapeutic strategies to prevent or treat deleterious consequences of cardiac hypertrophy, such as heart failure.

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