Jpn. J. Pharmacol. **80**, 97–102 (1999)

REVIEW —Current Perspective—

Activation of Mitogen-Activated Protein Kinases in Cardiovascular Hypertrophy and Remodeling

Shokei Kim and Hiroshi Iwao

Department of Pharmacology, Osaka City University Medical School, 1–4–3 Asahimachi, Abeno-ku, Osaka 545–8585, Japan

Received December 14, 1998

ABSTRACT—Extracellular signal-regulated kinases (ERKs) and c-jun NH_2-terminal kinases (JNKs), which belong to the family of mitogen-activated protein kinases (MAPKs), play a key role in the regulation of cell growth or apoptosis or various gene expressions. In spite of the critical importance of MAPKs for cell function in vitro, the role of MAPKs in the pathophysiology of the cardiovascular system in vivo is poorly understood. Recently, we have examined the activities of MAPKs in various cardiovascular disease models. JNKs activity is chronically enhanced in cardiac hypertrophy of hypertensive rats or angiotensin II-infused rats, which is followed by the increase in activator protein-1 (AP-1) activity composed of c-Fos and c-Jun proteins. In chronic hypertensive rats, vascular ERKs and JNKs activities are continuously increased compared with normotensive rats, with the development of vascular thickening. Furthermore, balloon injury rapidly and transiently activates vascular ERKs and JNKs, followed by the activation of AP-1. This activation of ERKs and JNKs in injured artery is in part mediated by angiotensin AT1 receptor. Thus, the enhanced activation of JNKs or ERKs occurs in various cardiovascular disease models, supporting the notion that MAPKs may be a useful target for treatment of cardiovascular hypertrophy and remodeling.

Keywords: Cardiovascular disease, Angiotensin II, Activator protein-1, Gene expression

Extracellular stimuli, including mechanical stretch, G protein-coupled receptor agonists, growth factors, cytokines and stresses, cause a wide variety of cellular responses such as cellular phenotypic changes, growth, apoptosis, migration and gene expressions. The first important molecular event involved in cellular responses is the activation of intracellular signal transduction pathways, particularly the activation of protein kinases. Mitogen-activated protein kinases (MAPKs), which are protein serine/threonine kinases, are regarded as some of the most important protein kinases responsible for cellular responses. MAPKs, composed of extracellular signal-regulated kinases (ERKs), c-jun NH_2-terminal kinases (JNKs) and p38-MAPKs, are activated by dual phosphorylation on both a threonine and a tyrosine residues, and they play critical roles in cell differentiation, growth and apoptosis and the regulation of various transcription factors and gene expressions (1–3) (Fig. 1). A growing body of in vitro evidence on cultured cells show that MAPKs may be implicated in cardiovascular cellular responses induced by external stimuli (4, 5). Recently, we have determined the activities of MAPKs in various experimental cardiovascular and renal diseases, and we obtained the results that the activation of ERKs and JNKs was enhanced in cardiovascular and glomerular tissues of various experimental disease models, supporting the notion that ERKs and JNKs may be responsible for the pathophysiology of cardiovascular and renal diseases (Table 1) (6–11). Furthermore, JNKs or p38-MAPKs in the heart are activated by ischemia/reperfusion (12), suggesting the involvement of these MAPKs in ischemia/reperfusion injury. Thus, the detailed investigations on the significance of MAPKs in these experimental models are necessary to elucidate the molecular and cellular mechanism of cardiovascular diseases. In this review, we will discuss the possible roles of MAPKs in cardiovascular hypertrophy and remodeling in vivo.

Differential activation and function of MAPKs

The cascade of ERKs (13), the most characterized of the three subfamilies of MAPKs, are principally activated by mechanical stretch, G protein-coupled receptor ago-

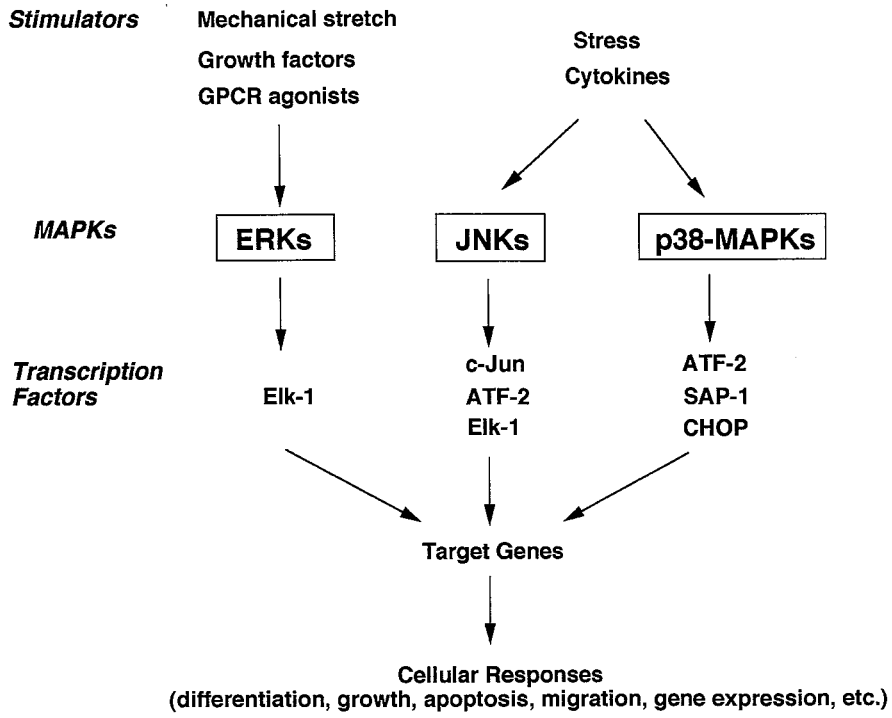

Fig. 1. The pathway of three subfamilies of mitogen-activated protein kinases. GPCR, G protein-coupled receptor.

nists (angiotensin II (Ang II), endothelin or α-adrenergic agonists, etc.), or stimulation of receptors with intrinsic tyrosine kinase activity. Unlike the activation of ERKs predominantly by mitogenic stimuli, JNKs (14) and p38-MAPKs (15) are activated mainly by cellular stresses such as inflammatory cytokines, ischemia, oxidative stress, osmotic shock, heat shock, ATP-depleting agent and endotoxin. As the upstream cascades of MAPKs and the regulation of transcription factors by MAPKs are described in detail in recent excellent reviews (4, 5), we will describe them only briefly in this review. ERKs, JNKs and p38-MAPKs are activated by the dual phosphorylation of a Thr-Xaa-Tyr motif by dual-specificity MAPKs kinases (MKKs), although these three subfamilies of MAPKs are predominantly activated by different MKKs. ERKs are activated by MKK1 and MKK2, JNKs are mainly activated by MKK7, while p38-MAPKs are activated by MKK3 and MKK6. Downstream targets are also different among the three MAPKs (Fig. 1). ERKs increase the enzymatic activity of cytoplasmic phospholipase A_2 by phosphorylation of its Ser505, leading to the increase in the release of arachidonic acid and the formation of lysophospholipids. Furthermore, ERKs phosphorylate Elk-1, a ternary complex factor, which forms a complex with serum response factor and together binds to the promoter of numerous genes such as *c-fos* containing the serum response element. Therefore, phosphorylation and activation of Elk-1 by ERKs lead to the increased induction of *c-fos* mRNA (16). Because c-Fos protein is an important component of transcription factor activator pro-

Table 1. Enhanced activation of ERKs and JNKs in experimental models of cardiovascular hypertrophy and remodeling, and glomerular diseases

Experimental models (References)	
Ang II-induced hypertension (11)	Cardiac JNKs activation precedes cardiac hypertrophy
Stroke-prone SHR (7)	JNKs and ERKs activities are chronically increased in the heart and aorta
DS rat (8)	Aortic ERKs activities are chronically increased by salt loading
Balloon injury (9)	Both JNKs and ERKs are rapidly and transiently activated, partially mediated by AT1 receptor
Glomerulonephritis (10)	Both JNKs and ERKs are activated in glomerular tissues; this activation is suppressed by prednisolone

SHR, spontaneously hypertensive rats; DS rat, Dahl salt-sensitive hypertensive rat.

tein-1 (AP-1), the activation of ERKs is thought to cause the activation of AP-1 (16). JNKs and p38-MAPKs phosphorylate different transcription factors from ERKs. JNKs are the predominant c-Jun kinases and readily phosphorylate c-Jun at Ser 63/73 (14). JNKs also phosphorylate the N-terminal transcriptional activation domain of activating transcription factor-2 (ATF-2), leading to its increased transcriptional activating activity (17). p38-MAPKs activate ATF-2, serum response factor accessory protein-1 (SAP-1) and CHOP (17). However, the downstream target genes of these three MAPKs remain to be determined.

Controversial findings on the role of MAPKs in cardiac myocyte hypertrophy in vitro

The role of ERKs, JNKs and p38-MAPKs in cardiac myocyte hypertrophy has been examined under in vitro conditions, mainly using neonatal rat cardiac myocytes. Recent work, investigating the effects of antisense oligodeoxynucleotide directed against ERKs on the phenylephrine-induced hypertrophic response in cultured rat cardiac myocytes, demonstrate that ERKs are responsible for the development of hypertrophy (18). On the other hand, other studies do not support the important contribution of ERKs to the cardiac myocyte hypertrophic response (5, 19, 20). The activation of p38 in neonatal rat cardiac myocytes, by transfection with MKK6, augments cell size, enhances atrial natriuretic factor (ANF) and skeletal α-actin promoter activities and elicits sarcomeric organization, supporting the important role of p38 in myocardial cell hypertrophy (21). In more recent work, specific activation of the JNK pathway in neonatal rat cardiac myocytes with the infection of recombinant adenoviral vectors expressing MKK7 induces characteristic features of hypertrophy, including an increase in cell size, elevated expression of ANF and induction of sarcomere organization, supporting the notion that JNK may play an active role in the development of cardiac myocyte hypertrophy (22). Thus, in contrast to the important role of p38-MAPKs and JNKs in apoptosis observed in PC-12 cells (3), the above reports suggest that p38-MAPKs or JNKs activations may cause cardiac myocyte hypertrophy rather than apoptosis (21, 22). However, co-activation of JNKs by MKK7 with p38-MAPKs by MKK6 in neonatal rat cardiac myocytes leads to an induction of cytopathic responses and suppression of JNKs-induced hypertrophic responses (22), suggesting that p38-MAPKs may contribute to myocyte apoptosis rather than hypertrophy. Thus, there exist conflicting in vitro data on the role of three families of MAPKs in the cardiac myocyte hypertrophic response. The in vivo investigation must be awaited to conclude the exact role of each subfamily of MAPKs in cardiac myocyte hypertrophy.

MAPKs in experimental cardiac hypertrophy and remodeling

Stroke-prone spontaneously hypertensive rats (SHRSP) are well established to be a useful model to examine the pathophysiology of not only hypertension but also cardiac hypertrophy and remodeling. To examine the possible contribution of MAPKs to cardiac hypertrophy and remodeling, we have determined cardiac MAPKs activities in SHRSP and compared them with those in control normotensive Wistar-Kyoto rats (WKY) at various ages (7). In WKY, left ventricular ERKs and JNKs activities decrease with age. Left ventricular ERKs and JNKs activities in prehypertensive SHRSP do not differ from those in WKY, while these activities begin to increase in SHRSP from the phase of mild hypertension and remain increased until the establishment of cardiac hypertrophy. Thus, the enhanced activation of left ventricular ERKs and JNKs in SHRSP compared with WKY occurs throughout the development of cardiac hypertrophy. Regression of cardiac hypertrophy of SHRSP with imidapril (10 mg/kg/day), an angiotensin-converting enzyme inhibitor, is accompanied by decreases in left ventricular JNKs activities but not ERKs activities. Thus, the mechanism of the increased activities in SHRSP differs between ERKs and JNKs. JNKs, rather than ERKs, may be involved in the pathophysiology of cardiac hypertrophy and remodeling in SHRSP.

Ang II directly causes cardiac hypertrophy and remodeling in vivo as shown by the fact that continuous infusion of Ang II to rats causes cardiac hypertrophy and fibrosis via AT1 receptors, independently of a blood pressure-elevating effect (23, 24). We have examined the effect of Ang II infusion on cardiac MAPKs (11). Left ventricular JNKs and ERKs are significantly activated by Ang II infusion via the AT1 receptor. Interestingly, Ang II-induced cardiac activation of JNK in vivo occurs in a more sensitive manner than that of ERK. Furthermore, left ventricular hypertrophy, induced by chronic Ang II infusion, is preceded by the significant activation of JNK without ERK activation. Thus, as in the case of cardiac hypertrophy of SHRSP, JNK may participate in the Ang II-induced cardiac hypertrophic response in vivo.

Possible implication of JNKs in activation of AP-1 in cardiac hypertrophy and remodeling

Which downstream cascades JNKs activate in vivo must be clarified to determine the exact role of JNKs in cardiac hypertrophy. As described above, as JNKs are the predominant c-Jun kinase, JNKs are the potent activator of AP-1 (16). Therefore, we have examined cardiac AP-1 activity in SHRSP and Ang II-infused rats. Left ventricu-

lar AP-1 DNA binding activity is significantly increased by Ang II infusion via the AT1 receptor, following the activation of JNKs (11). This increased AP-1 activity is due to c-Fos and c-Jun proteins. Furthermore, left ventricular AP-1 activity is also enhanced in SHRSP compared with WKY (Y. Izumi et al., unpublished data). Thus, it is postulated that the activation of JNKs in Ang II-infused rats or SHRSP may lead to the activation of AP-1.

Postulated role of JNKs/AP-1 cascade in cardiac gene expressions

Cardiac hypertrophy and remodeling, which play a central role in the development of various cardiac diseases and heart failure, are characterized not only by the increase in cell size but also by the significant changes in various cardiac performance-related gene expressions such as contractile proteins and extracellular matrix components (Table 2) (25, 26). AP-1 importantly regulates the expression of various genes by binding the AP-1 consensus sequence present in their promoter region (16). Interestingly, the fetal phenotype of cardiac genes such as skeletal α-actin and ANF and cardiac fibrosis-associated genes such as transforming growth factor-β1 (TGF-β1) and collagen type I have an AP-1 responsive sequence in their promoter region (16). Indeed, in cultured cells, AP-1 activation has been demonstrated to lead to the increased promoter activity of skeletal α-actin and TGF-β1. Furthermore, we have previously reported that the expression of the above mentioned cardiac genes is significantly enhanced in SHRSP and Ang II-infused rats (24, 27). These results suggest that JNK/AP-1 cascade may be involved in the increased expression of ANF, skeletal α-actin, TGF-β1 and collagen type I in cardiac hypertrophy (Fig. 2). However, the investigation on the effect of a dominant interfering mutant of JNKs or components of AP-1 such as c-Jun on cardiac gene expressions in vivo is essential to demonstrate our suggestion.

Table 2. General characteristics of cardiac hypertrophy and remodeling

Cardiac myocyte hypertrophy
Decreased gene expression of α-MHC (adult phenotype of gene)
Increased gene expression of fetal phenotype of genes
β-MHC, skeletal α-actin, ANF, etc.
Increased gene expression of extracellular matrix components
fibronectin, collagen types I, III and IV, laminin, etc.
Interstitial collagen deposition

MHC, myosin heavy chain; ANF, atrial natriuretic factor.

Activation of MAPKs in rat balloon-injured artery and role of angiotensin AT1 receptor

Vascular remodeling is characterized by an active process of structural changes involving cellular responses such as cell growth, cell death, cell migration, and accumulation or degradation of extracellular matrix; and it plays a key role in the pathophysiology of various vascular diseases (28). As shown by the findings on balloon injuy-induced vascular remodeling in vivo (28, 29), numerous gene expressions, such as proto-oncogenes, growth factors or extracellular matrices, are activated in the onset and development of vascular remodeling. However, the signal transduction pathway underlying the changes in gene expression is unclear. Recently, we have obtained evidence that both JNKs and ERKs are rapidly and transiently activated in rat balloon-injured artery (9). Furthermore, accumulating evidence on the in vivo effects of AT1-receptor antagonists indicates that Ang II, via AT1 receptors, plays a crucial role in the development of neointimal formation induced by balloon injury (23, 29). To examine the possible contribution of the AT1 receptor to ERKs and JNKs activation in balloon-injured artery, we have examined the effects of an AT1-receptor antagonist and have found that the AT1-receptor antagonist significantly inhibits the activation of ERKs and JNKs in injured artery, demonstrating that Ang II, via the AT1 receptor, is responsible for balloon injury-induced arterial ERKs and JNKs activation (9). Furthermore, arterial ERKs and JNKs activation is followed by the increase in c-fos and c-jun mRNAs and AP-1 activitiy, which is also

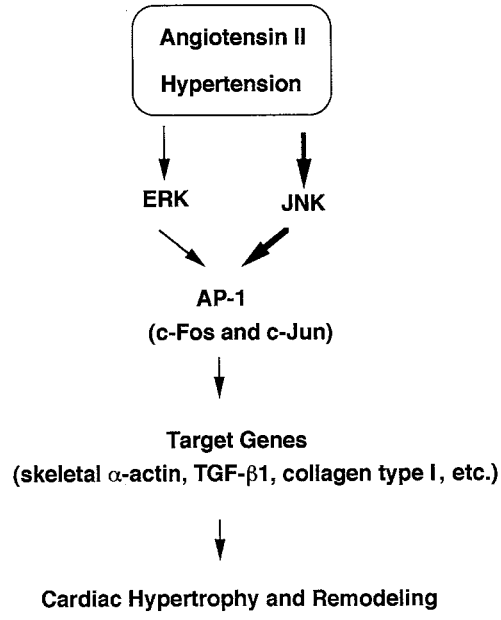

Fig. 2. Proposed role of ERKs and JNKs in cardiac hypertrophy and remodeling.

significantly suppressed by an AT1-receptor antagonist (29). Therefore, it is likely that AT1-receptor-mediated activation of ERKs and JNKs in injured artery may lead to the activation of AP-1.

Chronic activation of ERKs and JNKs in hypertensive vascular remodeling

We have examined vascular MAP kinase activities in hypertensive rats in vivo (8). Hypertension in Dahl salt-sensitive rats (DS rats), induced by a high-salt diet, is accompanied by the increased activity of aortic ERKs and JNKs, suggesting the involvement of hypertension in the activation of ERKs and JNKs. Of note, the sustained activation of aortic ERKs in DS rats is longer than JNKs. To examine whether the chronic increase in aortic ERKs and JNKs are events specific to hypertensive DS rats, we have also examined aoritc ERKs and JNK in SHRSP, which spontaneously develop hypertension, without salt loading. As in the case of DS rats, the development of hypertension in SHRSP is accompanied by the progressive increase in aortic ERKs activities. Thus, chronic hypertension leads to the sustained increase in aortic ERKs and JNKs activities. ERKs may be involved in the process of vascular remodeling in chronic hypertension. It remains to be elucidated why the increase in ERKs activities is not transient but chronic in hypertensive models.

Conclusions and future directions

As described above, the activation of ERKs and JNKs is increased in acute or chronic phase of various experimental cardiovascular and renal diseases. Taken together with accumulating in vitro evidence that MAPKs play key roles in cellular responses including cell proliferation, apoptosis or gene expression, our in vivo data (6–11) support the notion that ERKs and JNKs may be responsible for the pathophysiology of cardiovascular and renal diseases. Unfortunately, at present, there are no available specific and potent pharmacological inhibitors of MAPKs for in vivo experiments. Therefore, to elucidate the in vivo role of MAPKs in the pathophysiology of cardiovascular hypertrophy and remodeling, the most powerful approaches are to create transgenic animals expressing MAPKs in cardiovascular tissues in a specific manner or to examine the effect of in vivo gene transfer of dominant interfering mutants of MAPKs on various cardiovascular disease models. These strategies may allow us to elucidate the in vivo role of MAPKs in cardiovascular diseases.

Acknowledgments

This work was in part supported by Grants-in-Aid for Scientific Research 09670101 and 09470527 from the Ministry of Education, Science, Sports and Culture.

REFERENCES

1 Davis RJ: The mitogen-activated protein kinase signal transduction pathway. J Biol Chem **268**, 14553–14556 (1993)
2 Davis RJ: MAPKs: new JNK expands the group. Trends Biochem Sci **19**, 470–473 (1994)
3 Xia Z, Dickens M, Raingeaud J, Davis RJ and Greenberg ME: Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science **270**, 1326–1331 (1995)
4 Force T, Pombo CM, Avruch JA, Bonventre JV and Kyriakis JM: Stress-activated protein kinases in cardiovascular disease. Circ Res **78**, 947–953 (1996)
5 Sugden P and Clerk A: "Stress-responsive" mitogen-activated protein kinases (c-Jun N-terminal kinases and p38 mitogen-activated protein kinases) in the myocardium. Circ Res **83**, 345–352 (1998)
6 Hamaguchi A, Kim S, Yano M, Yamanaka S and Iwao H: Activation of glomerular mitogen-activated protein kinases in angiotensin II-mediated hypertension. J Am Soc Nephrol **9**, 372–380 (1998)
7 Izumi Y, Kim S, Murakami T, Yamanaka S and Iwao H: Cardiac mitogen-activated protein kinase activities are chronically increased in stroke-prone hypertensive rats. Hypertension **31**, 50–56 (1998)
8 Kim S, Murakami T, Izumi Y, Yano M, Miura K, Yamanaka S and Iwao H: Extracellular signal-regulated kinase and c-Jun NH_2-terminal kinase activities are continuously and differentially increased in aorta of hypertensive rats. Biochem Biophys Res Commun **236**, 199–204 (1997)
9 Kim S, Izumi Y, Yano M, Hamaguchi A, Miura K, Yamanaka S, Miyazaki H and Iwao H: Angiotensin blockade inhibits activation of mitogen-activated protein kinases in rat balloon-injured artery. Circulation **97**, 1731–1737 (1998)
10 Seto M, Kim S, Yoshifusa H, Nakamura Y, Masuda T, Hamaguchi A, Yamanaka S and Iwao H: Effects of prednisolone on glomerular signal transduction cascades in experimental glomerulonephritis. J Am Soc Nephrol **9**, 1367–1376 (1998)
11 Yano M, Kim S, Izumi Y, Yamanaka S and Iwao H: Differential activation of cardiac c-Jun amino-terminal kinase and extracellular signal-regulated kinase in angiotensin II-mediated hypertension. Circ Res **83**, 752–760 (1998)
12 Bogoyevitch MA, Gillespie-Brown J, Ketterman AJ, Fuller SJ, Ben-Levy R, Ashworth A, Marshall CJ and Sugden PH: Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart. Circ Res **79**, 162–173 (1996)
13 Ray LB and Sturgill TW: Insulin-stimulated microtubule-associated protein kinase is phosphorylated on tyrosine and threonine in vivo. Proc Natl Acad Sci USA **85**, 3753–3757 (1988)
14 Hibi M, Lin A, Smeal T, Minden A and Karin M: Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. Genes Dev **7**, 2135–2148 (1993)
15 Han J, Lee JD, Bibbs L and Ulevitch RJ: A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science **265**, 808–811 (1994)
16 Karin M: The regulation of AP-1 activity by mitogen-activated protein kinases. J Biol Chem **270**, 16483–16486 (1995)
17 Whitmarsh AJ, Yang SH, Su MS, Sharrocks AD and Davis RJ:

Role of p38 and JNK mitogen-activated protein kinases in the activation of ternary complex factors. Mol Cell Biol **17**, 2360–2371 (1997)

18 Glennon PE, Kaddoura S, Sale EM, Sale GJ, Fuller SJ and Sugden PH: Depletion of mitogen-activated protein kinase using an antisense oligodeoxynucleotide approach downregulates the phenylephrine-induced hypertrophic response in rat cardiac myocytes. Circ Res **78**, 954–961 (1996)

19 Post GR, Goldstein D, Thuerauf DJ, Glembotski CC and Brown JH: Dissociation of p44 and p42 mitogen-activated protein kinase activation from receptor-induced hypertrophy in neonatal rat ventricular myocytes. J Biol Chem **271**, 8452–8457 (1996)

20 Thorburn J, Xu S and Thorburn A: MAP kinase- and Rho-dependent signals interact to regulate gene expression but not actin morphology in cardiac muscle cells. EMBO J **16**, 1888–1900 (1997)

21 Zechner D, Thuerauf DJ, Hanford DS, McDonough PM and Glembotski CC: A role for the p38 mitogen-activated protein kinase pathway in myocardial cell growth, sarcomeric organization, and cardiac-specific gene expression. J Cell Biol **139**, 115–127 (1997)

22 Wang Y, Su B, Sah VP, Brown JH, Han J and Chien KR: Cardiac hypertrophy induced by mitogen-activated protein kinase kinase 7, a specific activator for c-Jun NH_2-terminal kinase in ventricular muscle cells. J Biol Chem **273**, 5423–5426 (1998)

23 Timmermans PBMWM, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler R, Saye J and Smith R: Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol Rev **45**, 205–251 (1993)

24 Kim S, Ohta K, Hamaguchi A, Yukimura T, Miura K and Iwao H: Angiotensin II induces cardiac phenotypic modulation and remodeling in vivo in rats. Hypertension **25**, 1252–1259 (1995)

25 Morgan HE and Baker KM: Cardiac hypertrophy: mechanical, neural, and endocrine dependence. Circulation **83**, 13–25 (1991)

26 Schwartz K, Carrier L, Mercadier JJ, Lompre AM and Boheler KR: Molecular phenotype of the hypertrophied and failing myocardium. Circulation **87**, Suppl **VII**, VII-5–VII-10 (1993)

27 Kim S, Ohta K, Hamaguchi A, Yukimura T, Miura K and Iwao H: Effects of an AT_1 receptor antagonist, an ACE inhibitor and a calcium channel antagonist on cardiac gene expressions in hypertensive rats. Br J Pharmacol **118**, 549–556 (1996)

28 Gibbons GH and Dzau VJ: The emerging concept of vascular remodeling. N Engl J Med **330**, 1431–1438 (1994)

29 Kim S, Kawamura M, Wanibuchi H, Ohta K, Hamaguchi A, Omura T, Yukimura T, Miura K and Iwao H: Angiotensin II type I receptor blockade inhibits the expression of immediate-early genes and fibronectin in rat injured artery. Circulation **92**, 88–95 (1995)