The renin-angiotensin system and cardiac hypertrophy

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The heart develops left ventricular hypertrophy (LVH) in response to increased afterload in order to compensate for its wall stress and to maintain normal cardiac function.¹ Although the development of cardiac hypertrophy is itself an adaptive phenomenon, it is an important cause of increased morbidity and mortality.² Thus cardiac hypertrophy has recently attracted attention as an important risk factor influencing the prognosis of patients with hypertension. Recently, much data have been accumulated with regard to the molecular mechanisms of cardiac hypertrophy. Recent advances include demonstration of the existence of the local renin-angiotensin system in the heart³ and involvement of angiotensin II in the formation of cardiac hypertrophy.⁴

In this paper we examine signal transduction pathways in cardiac hypertrophy that are induced by stress, and how the relation between mechanical stress and the local renin-angiotensin system affects cardiac hypertrophy.

Signal transduction system for the development of cardiac hypertrophy

Intracellular signals are generally transduced into the nucleus through a protein kinase cascade of phosphorylation.⁵ What kind of kinase cascades are activated in hypertrophied cardiomyocytes?

Mitogen activated protein kinases (MAPKs)^{6,7} are serine/threonine protein kinases and can be activated by a variety of stimuli such as platelet derived growth factor, epidermal growth factor, insulin, and others. Activated MAPKs translocate into the nucleus and activate nuclear transcription factors such as *c-myc*, NF-IL6 and Elk-1,⁸ and 90 kDa ribosomal S6 kinase (p90^{rk}), which is a downstream enzyme of the MAPKs,⁶ and phosphorylates nuclear lamins.⁹ These observations suggest that both MAPKs and p90^{rk} play vital roles in transducing signals into the nucleus. The upstream activator of MAPKs is reported to be MAPK kinase (MAPKK),¹⁰⁻¹² which is a dual specificity protein kinase that phosphorylates MAPKs on both threonine and tyrosine residues within a conserved TEY motif.¹³ MAPKK is also activated by phosphorylation of two serine residues.¹⁴ More recently, Raf-1 kinase (Raf-1), which is the product of the Raf-1 proto-oncogene, has been shown to activate MAPKK.^{15,16} Therefore, these four kinases (Raf-1, MAPKK, MAPKs and p90^{rk}) are candidates involved in the acceleration of protein biosynthesis in the hypertrophied heart.

To test this hypothesis we examined the

activities of these kinases in stretched cardiomyocytes.¹⁷ We first examined whether mechanical stretch activates Raf-1 in cultured neonatal rat cardiomyocytes. Raf-1 was activated as early as one minute, with maximum activity at two minutes after stretch (fig 1). Raf-1 activity decreased progressively and returned to basal levels at 30 minutes after stretch. Next, we investigated whether stretching of myocytes activates MAPKK. Stretch for one to 10 minutes significantly increased MAPKK activity. MAPKK activity was maximally activated five minutes after stretch and gradually decreased thereafter. Kinase activity returned to control levels at 30 minutes after stretch. We measured MAPK activity using myelin basic protein containing gels. An increase in the phosphorylation levels of myelin basic protein induced by 42 kDa and 44 kDa MAPK. Activity returned to control levels at 30 minutes after stretch (fig 2). Preliminary data showed that stretching of myocytes for 10 minutes increases p90^{rk} activity.¹⁸ We investigated the time course of stretch induced p90^{rk} activation. Activation was not observed at two minutes after stretch. An increase in activity was detectable at five minutes. The activity peaked at 10 minutes, and was sustained at higher than basal levels for at least 30 minutes after stimulation. At 60 minutes the activity returned to basal levels.¹⁷

We have shown the signalling pathways evoked by mechanical stress in neonatal rat cardiac myocytes and that stretching of myocytes sequentially activates Raf-1, MAPKK, MAPKs, and p90^{rk}. It has been reported that MAPKs translocate into the nucleus upon activation and activate transcription factors which contain potential MAPK phosphorylation sites.⁸ We have previously reported that mechanical stretch induces expression of immediate early response genes such as *c-myc* and *c-fos* as an early event.¹⁹⁻²¹ MAPKs activated by stretch may play an

Figure 1 Raf-1 activation by stretch. Cardiac myocytes were stretched by 20% as previously described¹⁷ for the indicated periods of time. Raf-1 activity was assayed using recombinant MAPKK as a specific substrate.¹⁷ A representative autoradiogram of stretch-induced Raf-1 activation in cardiac myocytes is shown.

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Figure 2 Activation of MAPKs by stretch. Cardiac myocytes were stretched by 20% for the indicated periods of time. MAPKs were immunoprecipitated with anti-MAPK antibodies and myelin basic protein phosphorylation activity was analysed. A representative autoradiogram is shown. Upper bands represent 44 kDa MAPK and lower bands 42 kDa MAPK.

important role in the induction of these genes. p90^{rk} is also considered a mediator in the intracellular kinase cascade and nuclear events because it can phosphorylate nuclear lamins.⁹ Thus the MAPK signalling pathway induced by mechanical stress would play a role in stimulating gene expression and protein synthesis, which may lead to hypertrophy in cardiac myocytes.

Involvement of angiotensin II in the development of cardiac hypertrophy

In clinical studies, the degree of left ventricular hypertrophy is not necessarily related to the severity of hypertension. This suggests the existence of factors modifying the development of cardiac hypertrophy in response to hypertension. Angiotensin II has been shown to induce cardiac hypertrophy in cultured cardiomyocytes.^{22,23} An accumulating body of data suggests that the local renin-angiotensin system plays an important role in the formation of cardiac hypertrophy.⁴ All components of the renin-angiotensin system (that is, renin, angiotensinogen, angiotensin converting enzyme (ACE), and angiotensin II receptor) have been detected in the heart at both mRNA and protein levels.³ Many recent reports have also shown that the cardiac renin-angiotensin system is activated in experimental LVH induced by haemodynamic overload. Increases in angiotensinogen and ACE mRNAs have been reported in the hypertrophied left ventricle of rats.²⁴ In addition, suppressor doses of ACE inhibitors can cause regression of cardiac hypertrophy with no change in systemic blood pressure.²⁵ ACE inhibitors also prevent an increase in left ventricular mass produced by abdominal aortic constriction without any change in afterload or plasma renin activity.²⁶ Moreover, it is known that there is a strong similarity between the signalling pathways induced by mechanical load and by angiotensin II: both pathways accelerate phosphatidyl inositol turnover and activate protein kinase C, which increases the activity of MAPKs. These signals lead to enhanced gene expression and protein synthesis in cardiomyocytes.^{17,18,20,23,27} Therefore, angiotensin II may

act to promote the hypertrophic growth of the cardiomyocytes by an autocrine or paracrine mechanism in stretch loaded cardiomyocytes.

Does angiotensin II directly mediate cardiac hypertrophy produced by pressure overload? To answer this question, cardiomyocytes were pretreated with CV-11974 (a specific antagonist of the type 1 angiotensin II receptor) and mechanically stressed.^{17,23,28} Stretch-induced Raf-1 activation was partially inhibited by pretreatment with CV-11974 (fig 3), indicating that only a part of Raf-1 activation by mechanical stretch is dependent on secreted angiotensin II. The involvement of angiotensin II in stretch induced MAPK activation was also investigated by kinase assays using myelin basic protein containing gel.¹⁸ Stretch-induced MAPK activation was suppressed by 60% when cardiomyocytes were pretreated with CV-11974 (fig 3).²³ Pretreatment with the type 2 angiotensin II receptor specific antagonist, PD123319, had no effect on stretch induced activation of Raf-1 or MAPKs (data not shown). These results suggest that there are two signal transduction pathways for mechanical stress: an angiotensin II dependent (through type 1 receptor) and an angiotensin II independent pathway. Pretreatment with CV-11974 also partially inhibited the transient expression of *c-fos* expression induced by mechanical stretch.²⁸ In further studies, cardiomyocytes in culture were stretched and the conditioned culture medium was transferred to culture non-stretched cardiomyocytes. The stretch conditioned medium increased MAPK activities of non-stretched cardiomyocytes significantly and activation was completely suppressed by addition of CV-11974.²³ These results suggest that angiotensin II is the only secreted factor that activates hypertrophic events in stretched cardiac myocytes. However, stretch-induced activation of Raf-1 and MAPKs was only partially inhibited by pretreatment with a type 1 angiotensin II

Figure 3 Partial dependence of stretch induced Raf-1 and MAPK activation on angiotensin II (ANG II). Cardiac myocytes were stretched for 2 min (Raf-1) or 8 min (MAPKs) without or with pretreatment by CV-11974. Raf-1 and MAPK activities were measured as in fig 1 and fig 2. Values represent the mean, error bars = SEM, from four independent experiments. Total activities of the 42 kDa and 44 kDa MAPKs are shown.

receptor antagonist. Since the amount of the antagonist used in the present study was sufficient to suppress the maximum effect of angiotensin II,²³ secreted angiotensin II induced by stretch may only be partially involved in Raf-1 and MAPK activation.

We also measured angiotensin II in "stretch conditioned media" by radioimmunoassay using specific antibodies. An increase in angiotensin II concentration was detected in some but not all samples. The concentration of angiotensin II in the culture medium of non-stretched myocytes was below three pM. Using an in vitro model similar to ours, other investigators have also reported that mechanical stretch causes direct secretion of angiotensin II from cardiomyocytes and that stretch induced hypertrophic responses are completely dependent on secreted angiotensin II.²⁹ Thus stretch itself may stimulate MAPK activity, and the autocrine or paracrine mechanisms of angiotensin II may contribute in part to the development of stretch induced cardiac hypertrophy.

Conclusions

In conclusion, angiotensin II plays a critical role in MAPK activation in cardiac myocytes as an "exogenous" factor of mechanical stress. There are also "endogenous" factors in cardiac myocytes during mechanical stress. In other words, mechanical stress itself may evoke a hypertrophic response, and secreted angiotensin II may evoke hypertrophy promoting signals and amplify the response. We cannot rule out the possibility of contamination by cardiac non-myocytes, especially endothelial cells which may contribute to the production of angiotensin II, because it is known that a large part of ACE activity in the heart is localised to endothelial cells.³⁰ Our studies using an in vitro system of stretching cultured myocardial cells suggest the following sequence of protein kinase phosphorylation cascade: external mechanical stress → Raf-1 → MAPKK → MAPKs → p90^{rk}. We also showed that a type 1 angiotensin II receptor antagonist inhibits intracellular signals associated with cardiomyocyte hypertrophy, and that angiotensin II is directly secreted from stretched cardiac myocytes. This suggests that the local renin-angiotensin system plays a crucial role in the development of pressure overloaded cardiac hypertrophy. However, further investigation is necessary to clarify the precise molecular mechanisms by which mechanical stress induces biochemical signals and stimulates angiotensin II secretion.

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- Morgan HE, Gordon EE, Kira Y, Chua HL, Russo LA, Peterson CJ, et al. Biochemical mechanisms of cardiac hypertrophy. *Annu Rev Physiol* 1987;49:533-43.
- Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990;322:1561-6.
- Lindpaintner K, Ganten D. The cardiac renin-angiotensin system. An appraisal of present experimental and clinical evidence. *Circ Res* 1991;68:905-21.
- Baker KM, Booz GW, Dostal DE. Cardiac actions of angiotensin II: role of an intracardiac renin-angiotensin system. *Annu Rev Physiol* 1992;54:227-41.
- Cantley LC, Auger KR, Carpenter C, Duckworth B, Graziani A, Kapeller R, et al. Oncogenes and signal transduction (published erratum appears in *Cell* 1991;65: following 914). *Cell* 1991;64:281-302.
- Sturgill TW, Ray LB, Erikson E, Maller JL. Insulin-stimulated MAP-2 kinase phosphorylates and activates ribosomal protein S6 kinase II. *Nature* 1988;334:715-8.
- Ahn NG, Weiel JE, Chan CP, Krebs EG. Identification of multiple epidermal growth factor-stimulated protein serine/threonine kinases from Swiss 3T3 cells. *J Biol Chem* 1990;265:11487-94.
- Davis RJ. The mitogen-activated protein kinase signal transduction pathway. *J Biol Chem* 1993;268:14553-6.
- Ward GE, Kirschner MW. Identification of cell cycle-regulated phosphorylation sites on nuclear lamin C. *Cell* 1990;61:561-77.
- Gomez N, Cohen P. Dissection of the protein kinase cascade by which nerve growth factor activates MAP kinases. *Nature* 1991;353:170-3.
- Ahn NG, Seger R, Bratlen RL, Diltz CD, Tonks NK, Krebs EG. Multiple components in an epidermal growth factor-stimulated protein kinase cascade. In vitro activation of a myelin basic protein/microtubule-associated protein 2 kinase. *J Biol Chem* 1991;266:4220-7.
- Matsuda S, Kosako H, Takenaka K, Moriyama K, Sakai H, Akiyama T, et al. Xenopus MAP kinase activator: identification and function as a key intermediate in the phosphorylation cascade. *EMBO J* 1992;11:973-82.
- Nishida E, Gotoh Y. The MAP kinase cascade is essential for diverse signal transduction pathways. *Trends Biochem Sci* 1993;18:128-31.
- Zheng CF, Guan KL. Activation of MEK family kinases requires phosphorylation of two conserved Ser/Thr residues. *EMBO J* 1994;13:1123-31.
- Kovacina KS, Yonezawa K, Brautigan DL, Tonks NK, Rapp UR, Roth RA. Insulin activates the kinase activity of the Raf-1 proto-oncogene by increasing its serine phosphorylation. *J Biol Chem* 1990;265:12115-8.
- Blackshear PJ, Haupt DM, App H, Rapp UR. Insulin activates the Raf-1 protein kinase. *J Biol Chem* 1990;265:12131-4.
- Yamazaki T, Komuro I, Kudoh S, Zou Y, Shiojima I, Mizuno T, et al. Mechanical stress activates protein kinase cascade of phosphorylation in neonatal rat cardiac myocytes. *J Clin Invest* 1995;96:438-46.
- Yamazaki T, Tobe K, Hoh E, Maemura K, Kaida T, Komuro I, et al. Mechanical loading activates mitogen-activated protein kinase and S6 peptide kinase in cultured rat cardiac myocytes. *J Biol Chem* 1993;268:12069-76.
- Komuro I, Kurabayashi M, Takaku F, Yazaki Y. Expression of cellular oncogenes in the myocardium during the developmental stage and pressure-overloaded hypertrophy of the rat heart. *Circ Res* 1988;62:1075-9.
- Komuro I, Kaida T, Shibazaki Y, Kurabayashi M, Katoh Y, Hoh E, et al. Stretching cardiac myocytes stimulates protooncogene expression. *J Biol Chem* 1990;265:3595-8.
- Komuro I, Shibazaki Y, Kurabayashi M, Takaku F, Yazaki Y. Molecular cloning of gene sequences from rat heart rapidly responsive to pressure overload. *Circ Res* 1990;66:979-85.
- Katoh Y, Komuro I, Shibazaki Y, Yamaguchi H, Yazaki Y. Angiotensin II induces hypertrophy and oncogene expression in cultured cardiac myocytes. *Circulation* 1989;80(suppl II):II-450.
- Yamazaki T, Komuro I, Kudoh S, Zou Y, Shiojima I, Mizuno T, et al. Angiotensin II partly mediates mechanical stress-induced cardiac hypertrophy. *Circ Res* 1995;77:258-65.
- Schunkert H, Dzau VJ, Tang SS, Hirsch AT, Apstein CS, Lorell BH. Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy. Effects on coronary resistance, contractility, and relaxation. *J Clin Invest* 1990;86:1913-20.
- Linz W, Scholkens BA, Ganten D. Converting enzyme inhibition specifically prevents the development and induces regression of cardiac hypertrophy in rats. *Clin Exp Hypertens* 1989;11:1325-50.
- Baker KM, Chermis MI, Wixson SK, Aceto JF. Renin-angiotensin system involvement in pressure-overload cardiac hypertrophy in rats. *Am J Physiol* 1990;259:H324-32.
- Komuro I, Katoh Y, Kaida T, Shibazaki Y, Kurabayashi M, Hoh E, et al. Mechanical loading stimulates cell hypertrophy and specific gene expression in cultured rat cardiac myocytes. Possible role of protein kinase C activation. *J Biol Chem* 1991;266:1265-8.
- Kojima M, Shiojima I, Yamazaki T, Komuro I, Zou Z, Wang Y, et al. Angiotensin II receptor antagonist TCV-116 induces regression of hypertensive left ventricular hypertrophy in vivo and inhibits the intracellular signaling pathway of stretch-mediated cardiomyocyte hypertrophy in vitro. *Circulation* 1994;89:2204-11.
- Sadoshima J, Xu Y, Slayter HS, Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell* 1993;75:977-84.
- Yamada H, Fabris B, Allen AM, Jackson B, Johnston CI, Mendelsohn AO. Localization of angiotensin converting enzyme in rat heart. *Circ Res* 1991;68:141-9.