Biochemical and Biophysical Research Communications **259,** 8–14 (1999)
Article ID bbrc.1999.0720, available online at http://www.idealibrary.com on IDEAL®

Pulsatile Stretch Activates Mitogen-Activated Protein Kinase (MAPK) Family Members and Focal Adhesion Kinase ($p125^{FAK}$) in Cultured Rat Cardiac Myocytes

Yoshinori Seko,*,†,‡,[1] Yuko Seko,§ Naoyuki Takahashi,* Kazuyuki Tobe,¶ Takashi Kadowaki,¶ and Yoshio Yazaki*

**Department of Cardiovascular Medicine and ¶Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo, Bunkyo-ku, Tokyo; †Department of Immunology, School of Medicine, Juntendo University, Bunkyo-ku, Tokyo; ‡Institute for Adult Diseases, Asahi Life Foundation, Shinjuku-ku, Tokyo; and §Department of Ophtalmology, School of Medicine, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan*

Received April 16, 1999

Recently, we demonstrated that pulsatile mechanical stretch induced rapid secretion of vascular endothelial growth factor (VEGF) by cultured rat cardiac myocytes *in vitro*. To investigate whether pulsatile stretch activates intracellular signaling in cardiac myocytes, we examined the activation of mitogen-activated protein kinase (MAPK) family members and focal adhesion kinase ($p125^{FAK}$) in cultured rat cardiac myocytes. We found that pulsatile stretch rapidly phosphorylated p44/p42 MAPKs (extracellular signal-regulated protein kinase [ERK] 1/2), stress-activated protein kinase (SAPK), p38MAPK, and $p125^{FAK}$. The stretch-induced activation of ERKs was at least partly mediated by VEGF, which was shown to be induced by transforming growth factor (TGF)-β, and was also partly dependent on tyrosine kinases as well as protein kinase C (PKC). These data provide the direct evidence that pulsatile stretch can activate intracellular signaling in cardiac myocytes and that this was at least partly mediated by VEGF, which may play a role in cardiac adaptation to mechanical overload.

Cardiac myocytes are known to express various genes coding for growth factors, cytokines and so on, in response to mechanical overload to adapt to this external stress, which may lead to myocardial hypertrophy (1-4). It has been shown that pulsatile myocardial stretch as well as myocardial ischemia (hypoxia) *in vivo* markedly enhanced vascular endothelial growth factor (VEGF) mRNA level in the heart (5-7). We previously reported that serum levels of VEGF were markedly increased in patients with acute myocardial infarction (8). This strongly suggests that cardiac myocytes release a large amount of VEGF in response to acute ischemia and that most of the tissue cells including cardiac myocytes are exposed to high levels of VEGF in such a condition as acute myocardial infarction. Recently, we have demonstrated that pulsatile mechanical stretch induced rapid secretion of VEGF by cultured rat cardiac myocytes and mRNA expression of VEGF and VEGF receptors in the cardiac myocytes *in vitro* (9). We also found that the stretch-induced secretion of VEGF was at least in part mediated by transforming growth factor (TGF)-β. These data provide the direct evidence that mechanical overload itself can induce VEGF secretion by cardiac myocytes, which may play a role in ameliorating the relative myocardial hypoxia.

As for the intracellular signaling cascades activated by hypoxia in cultured rat cardiac myocytes, we previously reported that hypoxia caused rapid activation of RAS/mitogen-activated protein kinase (MAPK; also called extracellular signal-regulated protein kinase [ERK]) pathway, two other stress-activated MAPK family members, stress-activated protein kinase (SAPK; also called Jun N-terminal kinase [JNK]) and p38MAPK, and Src family tyrosine kinases, $p60^{c\text{-}src}$ and $p59^{c\text{-}fyn}$ (10-12). In this study, because pulsatile mechanical stretch as well as hypoxia were shown to induce VEGF in cardiac myocytes, to investigate

[1] Address for correspondence: Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Fax: +81-3-5800-5139. E-mail: sekoyosh-tky@umin.ac.jp.

Abbreviations used: VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor-β; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated protein kinase; SAPK, stress-activated protein kinase; JNK, Jun N-terminal kinase; FAK, focal adhesion kinase; PKC, protein kinase C; Tyr, tyrosine; Thr, threonine; mAb, monoclonal antibody; ECM, extracellular matrix.

0006-291X/99 $30.00

whether pulsatile stretch activates intracellular signaling in cardiac myocytes, we analyzed the activation of MAPK family members as well as focal adhesion-associated tyrosine kinase $p125^{FAK}$ in cultured rat cardiac myocytes *in vitro*. In addition, to investigate whether autocrine soluble factors are involved in the mechanism of stretch-induced signal transduction in cardiac myocytes, we examined the effects of neutralizing antibodies or receptor antagonists for several growth factors, known to be induced by mechanical stretch (4, 7, 13, 14), on stretch-induced activation of MAPK. Furthermore, to investigate the involvement of tyrosine kinases or protein kinase C (PKC), in stretch-induced signal transduction in cardiac myocytes, we also analyzed the effects of a tyrosine kinase inhibitor and a PKC inhibitor on stretch-induced activation of MAPK.

MATERIALS AND METHODS

Cell culture. Primary culture of ventricular cardiac myocytes were prepared from the neonatal rats as previously described (10). They were dispersed onto BioFlex collagen I culture plates coated with type-I collagen (Flexcell International Corporation, McKeesport, PA, USA) and cultured for 2 days until they were confluent.

Application of continuous pulsatile stretch. To study the effects of pulsatile stretch on VEGF secretion and expression by cardiac myocytes, and *in vitro* pulsatile stretch device was used as previously reported (9, 15). Cultured cardiac myocytes were serum-starved for 24 hr. After culture medium was replaced with a new one, the culture plates were placed on a computerized Flexercell Strain Unit gasketed baseplate in the incubator (Flexcell International Corporation) and subjected to pulsatile mechanical stretch (60 cycles per min, 15% elongation). Culture plates not subjected to pulsatile mechanical stretch were used as controls.

Western blot analyses for phosphorylation of ERKs, SAPKs, and p38MAPK. Cardiac myocytes were subjected to pulsatile stretch for the indicated time periods, then the culture media were aspirated immediately and cardiac myocytes were frozen in liquid nitrogen. The cells were lysed on ice with buffer A (25 mmol/L Tris/HCl [pH 7.6], 25 mmol/L NaCl, 1 mmol/L Na_3VO_4, 10 mmol/L sodium pyrophosphate, 10 nmol/L okadaic acid, 0.5 mmol/L EGTA, 1 mmol/L phenylmethylsulflonyl fluoride [PMSF]), and the cell lysates were centrifuged. The supernatants were suspended in Lemli's sample buffer. Aliquots of the samples were subjected to Western analyses using a rabbit polyclonal phospho-specific p44/p42MAPK (ERK) (Tyr204), SAPKs (Thr183/Tyr185), and p38MAPK (Thr180/Tyr182) antibody or a rabbit polyclonal control anti-p44/p42MAPK (ERK1/2), SAPKs, and p38MAPK antibody (New England Biolabs, Inc., Beverly, MA, USA), respectively. The antibody-antigen complexes were developed with chemiluminescence using alkalinephosphatase (Phototope-Star Detection Kit, New England Biolabs, Inc.). The intensity of phosphorylation at the 44/42 kD bands of ERKs, 54/46 kD bands of SAPKs, and 38 kD bands of p38MAPK was quantified by scanning densitometry.

Western blot analysis for phosphorylation of $p125^{FAK}$. Cardiac myocytes were subjected to pulsatile stretch for the indicated time periods, then they were frozen in liquid nitrogen and lysed on ice with buffer A and 1% Nonidet P-40. The cell lysates were centrifuged and the supernatants containing detergent soluble proteins were collected. The cell lysates were immunoprecipitated with a rabbit anti-$p125^{FAK}$ polyclonal antibody (16) along with protein G-Sepharose (Amersham Pharmacia Biotech AB, Uppsala, Sweden) at 4°C over night. The immunoprecipitates were washed once with lysis buffer and eluted by boiling in Lemli's sample buffer for 5 min. The eluted proteins were subjected to Western analysis using a mouse anti-phosphotyrosine monoclonal antibody (mAb). (4G10; Upstate Biotechnology Inc., Lake Placid, NY, USA). To confirm that equal amounts of $p125^{FAK}$ protein were immunoprecipitated in each reaction, the same membranes were stripped by incubating in a solution containing 100 mmol/L 2-mercaptoethanol, 2% SDS, and 62.5 mmol/L Tris-HCl (pH 6.8) for 30 min at 50°C, then blotted with the anti-$p125^{FAK}$ antibody. The antibody-antigen complexes were developed with chemiluminescence using alkalinephosphatase. The intensity of phosphorylation at the 125 kD bands of $p125^{FAK}$ was quantified by scanning densitometry.

Inhibition of VEGF, TGF-β, angiotensin II, and endothelin activities. Cultured cardiac myocytes were serum-starved for 24 hr, and preincubated with neutralizing anti-VEGF mAb (mouse IgG, 10 μg/ml; R & D systems Inc., Minneapolis, MN, USA), or neutralizing anti-TGF-β antibody (chicken IgY; R & D systems Inc.) (5 μg/ml) or control rat IgG (5 μg/ml; ICN Pharmaceuticals, Inc., Aurora, OH, USA), or angiotensin II (AT1) receptor antagonist (CV11974, 0.1 μM, Takeda Chemical Industries, Ltd., Osaka, Japan), or endothelin receptor antagonists (BQ-123, 22 μM for ET_A receptor and BQ-788, 1.2 μM for ET_B receptor; Calbiochem-Novabiochem Japan Ltd., Tokyo, Japan) for 1 hr, respectively. The cardiac myocytes were subjected to pulsatile stretch for 5 min, then we examined the effect of pulsatile stretch on the phosphorylation of ERKs by Western blot analysis. The intensity of phosphorylation at the 42/44 kD bands of ERKs was quantified by scanning densitometry.

Inhibition of tyrosine kinase and PKC activities. Genistein is a nonselective inhibitor of tyrosine kinase activity that competes with ATP for binding to the enzyme. Calphostin C is a highly potent and specific inhibitor of PKC. Treatment with 1 μmol/L calphostin C has been shown to completely inhibit PKC activity (17). To investigate the involvement of tyrosine kinases and PKC in the increased phosphorylation of ERKs, we pretreated cardiac myocytes with 185 μmol/L (50 μg/mL) genistein (Gibco BRL, Life Technologies, Inc., Grand Island, NY, USA), or 1 μmol/L calphostin C (Wako Pure Chemical Industries Ltd., Osaka, Japan), or both for 30 min, then examined the effect of pulsatile stretch on the phosphorylation of ERKs by Western blot analysis. The intensity of phosphorylation at the 42/44 kD bands of ERKs was quantified by scanning densitometry.

Statistical analysis. Unpaired t-test was used for comparison except for the analysis of phosphorylation of $p125^{FAK}$, in which paired t-test was used. Values are mean ± S.D. of one representative experiment. Values of $p < 0.05$ were considered significant.

RESULTS

Pulsatile stretch activates ERK1/2, SAPK, and p38MAPK in cardiac myocytes. To investigate whether pulsatile stretch activates MAPK family members in cardiac myocytes, we examined whether pulsatile stretch phosphorylates ERK1/2, SAPK, and p38MAPK. As shown in Figure 1 (A to C, upper and lower panels), pulsatile stretch rapidly and significantly ($p < 0.01$, respectively) phosphorylated ERK1/2, SAPK (p54/p46), and p38MAPK, respectively, indicating the activation of these kinases. The phosphorylation was led to a maximum level at about 5 min of stimulation. We confirmed that almost equal amounts of ERK1/2, SAPK, and p38MAPK protein were electrophoresed in each reaction by Western analysis using a control antibodies against these kinases

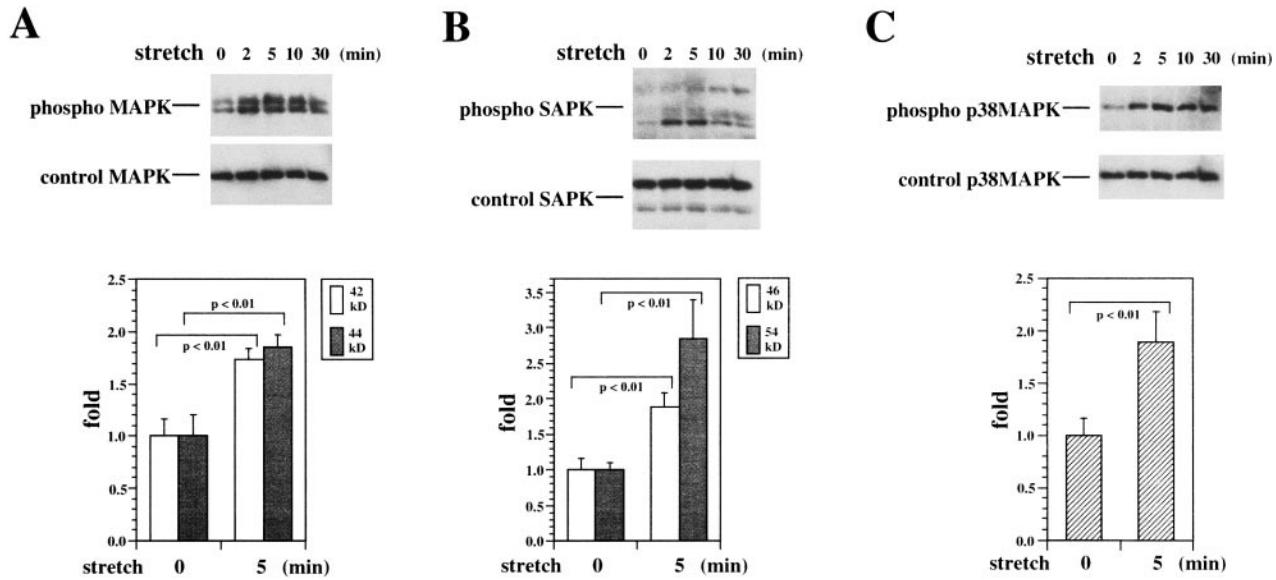

FIG. 1. Phosphorylation of p44/p42MAPK, p54/p46SAPK, and p38MAPK in cardiac myocytes subjected to pulsatile mechanical stretch. Serum-starved cardiac myocytes were subjected to pulsatile mechanical stretch (60 cycles per min, 15% elongation) for the indicated time periods and lysed in buffer A. The cell lysates were centrifuged, and the supernatants were subjected to Western analyses using a phospho-specific p44/p42MAPK (ERK) (A), p54/p46SAPK (B), or p38MAPK (C) antibody (upper panels) or a control anti-p44/p42MAPK (ERK) (A), -p54/p46SAPK (B), or -p38MAPK antibody (C) (middle panels). The antibody-antigen complexes were developed with chemiluminescence using alkalinephosphatase. The results shown are from one typical experiment. (A to C, lower panels) The results shown represent the (mean ± SD) form one typical experiment ($n = 3$). The control activity at 0 min is designated as 1.0. We performed at least three independent experiments.

(phosphorylation-state independent) (Figure 1, A to C, middle panels).

Pulsatile stretch activates p125^{FAK}. As shown in Figure 2A (upper panel) and Figure 2B, pulsatile stretch rapidly and significantly ($p < 0.05$) increased tyrosine phosphorylation of p125^{FAK}, indicating the activation of p125^{FAK}. The maximal phosphorylation of p125^{FAK} occurred at about 5 min of stimulation. We confirmed that almost equal amounts of p125^{FAK} protein were immunoprecipitated in each reaction (Figure 2A, lower panel).

Neutralization of VEGF or TGF-β activity significantly attenuated pulsatile stretch-induced activation of MAPK (ERK1/2) in cardiac myocytes. Next, to examine whether stretch-induced activation of intracellular signaling in cardiac myocytes is mediated by autocrine release of some soluble factors such as VEGF, TGF-β, angiotensin II, or endothelin, we analyzed the effect of neutralization or inhibition of the activity of these growth factors on pulsatile stretch-induced activation of MAPK (ERK1/2). As shown in Figure 3A (upper panel), pulsatile stretch apparently phosphorylated MAPK (ERK1/2) at 5 min of stimulation in control group, rat IgG-treated control group, angiotensin II antagonist-treated group, and endothelin antagonist-treated group. Whereas, pulsatile stretch failed to phosphorylate MAPK (ERK1/2) at 5 min of stimulation in neutralizing anti-VEGF mAb-treated group and neutralizing anti-TGF-β antibody-treated group. We confirmed that almost equal amounts of MAPK (ERK1/2) protein were electrophoresed in each reaction by Western analysis using a control anti-MAPK (ERK1/2) antibody (phosphorylation-state independent) (Figure 3A, lower panel). Figure 3B shows the fold induction of the phosphorylation of p44/p42MAPK (ERK1/2) at 5 min of stimulation as compared with that at 0 min in each group. As compared with the control group, pulsatile stretch-induced phosphorylation of p44 as well as p42MAPK was significantly attenuated in neutralizing anti-VEGF mAb-treated group and neutralizing anti-TGF-β antibody-treated group ($p < 0.05$, respectively). This indicates that VEGF as well as TGF-β may play a critical role in pulsatile stretch-induced activation of MAPK (ERK1/2) in cardiac myocytes.

Effects of tyrosine kinase inhibitor and PKC inhibitor on pulsatile stretch-induced phosphorylation of MAPK (ERK1/2) in cardiac myocytes. Furthermore, to examine whether pulsatile stretch-induced activation of MAPK (ERK1/2) is dependent on tyrosine kinase or PKC activity, the cardiac myocytes were pretreated with a tyrosine kinase inhibitor genistein, or PKC inhibitor calphostin C, or both for 30 min before stimulation. As shown in Figure 4A (upper panel), pulsatile stretch apparently phosphorylated MAPK (ERK1/2) at 5 min of stimulation in control group, whereas treatment with genistein or calphostin C moderately attenuated pulsatile stretch-induced

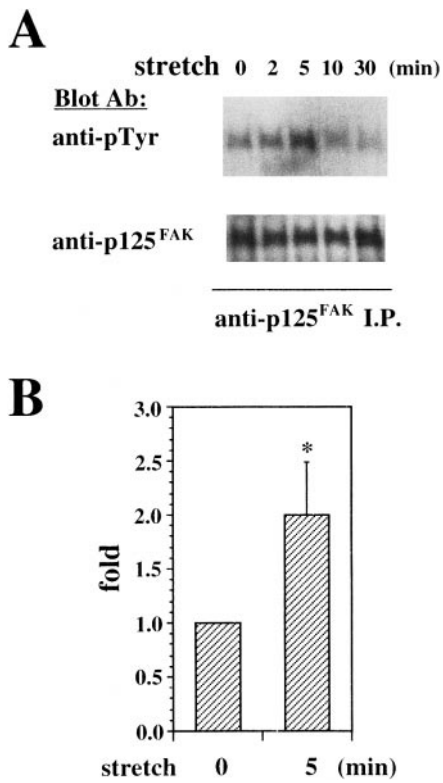

FIG. 2. Phosphorylation of p125^{FAK} in cardiac myocytes subjected to pulsatile mechanical stretch. Serum-starved cardiac myocytes were subjected to pulsatile mechanical stretch (60 cycles per min, 15% elongation) for the indicated time periods and lysed in buffer A and 1% Nonidet P-40. The cell lysates were immunoprecipitated with anti-p125^{FAK} antibody and Western blotted with anti-phosphotyrosine mAb (A, upper panel) or with the anti-p125^{FAK} antibody (A, lower panel). The antibody-antigen complexes were visualized by alkalinephosphatase reaction. The result shown represents one typical experiment. (B) The results shown represent the (mean ± SD) from one typical experiment ($n = 3$). The control activity at 0 min is designated as 1.0. We performed at least three independent experiments. (*$p < 0.05$ versus control [0 min]).

phosphorylation of MAPK (ERK1/2) at 5 min of stimulation. In addition, treatment with both genistein and calphostin C almost completely inhibited pulsatile stretch-induced phosphorylation of MAPK (ERK1/2) at 5 min of stimulation. We confirmed that almost equal amounts of MAPK (ERK1/2) protein were electrophoresed in each reaction by Western analysis using a control anti-MAPK (ERK1/2) antibody (phosphorylation-state independent) (Figure 4A, lower panel). Figure 4B shows the fold induction of the phosphorylation of p44/p42MAPK (ERK1/2) at 5 min of stimulation as compared with that at 0 min in each group. As compared with the control group, genistein significantly attenuated pulsatile stretch-induced phosphorylation of p42MAPK ($p < 0.01$) as well as p44MAPK ($p < 0.005$), and calphostin C also significantly attenuated pulsatile stretch-induced phosphorylation of p42MAPK ($p < 0.005$) as well as p44MAPK ($p < 0.001$). The effects of genistein and calphostin C on pulsatile stretch-induced phosphorylation of MAPK seem to be additive to some extent. In fact, the effect of (genistein plus calphostin C) for p42MAPK was significantly greater than that of genistein ($p < 0.05$) as well as that of calphostin C ($p < 0.001$), whereas no significant differences were seen among the effect of genistein, calphostin C, and (genistein plus calphostin C) for p44MAPK.

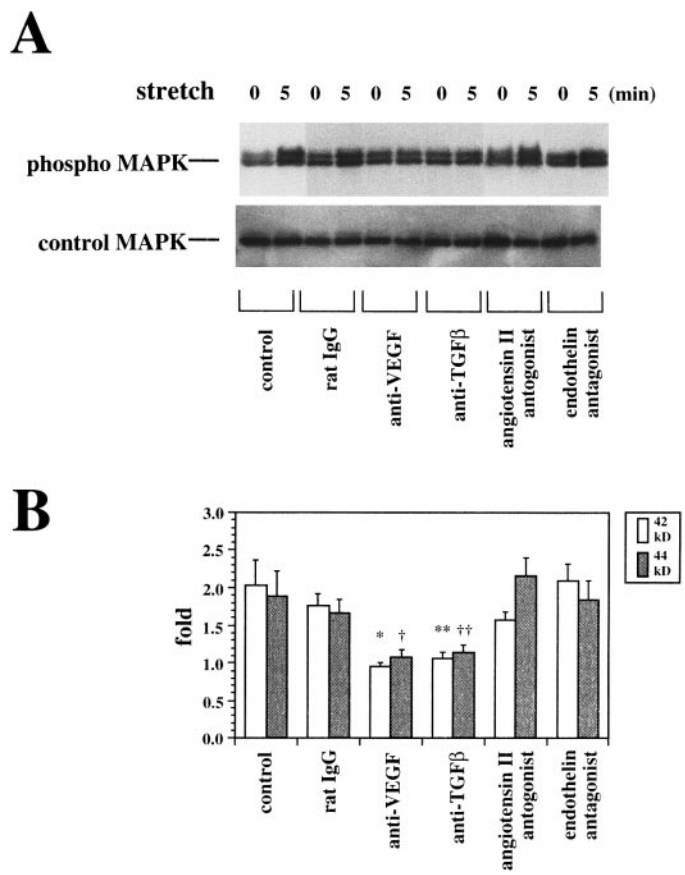

FIG. 3. Effects of neutralization or inhibition of VEGF, TGF-β, angiotensin II, or endothelin activity on phosphorylation of p44/p42MAPK in cardiac myocytes subjected to pulsatile mechanical stretch. Serum-starved cardiac myocytes were preincubated with control rat IgG, neutralizing anti-VEGF mAb, neutralizing anti-TGF-β antibody, angiotensin II receptor antagonist, or endothelin receptor antagonists for 1 h and then subjected to pulsatile mechanical stretch (60 cycles per min, 15% elongation) for 5 min. The cells were lysed in buffer A, and the cell lysates were subjected to Western analyses using a phospho-specific p44/p42MAPK (ERK) antibody (A, upper panel) or a control anti-p44/p42MAPK (ERK) antibody (A, lower panel). The antibody-antigen complexes were developed with chemiluminescence using alkalinephosphatase. The results shown are from one typical experiment. Panel B shows the fold induction of the phosphorylation of p44/p42MAPK at 5 min of stimulation as compared with that at 0 min. The results shown represent the (mean ± SD) from one typical experiment ($n = 3$). We performed at least three independent experiments. (p42MAPK, *$p < 0.05$ and **$p < 0.05$ versus control; p44MAPK, †$p < 0.05$ and ††$p < 0.05$ versus control).

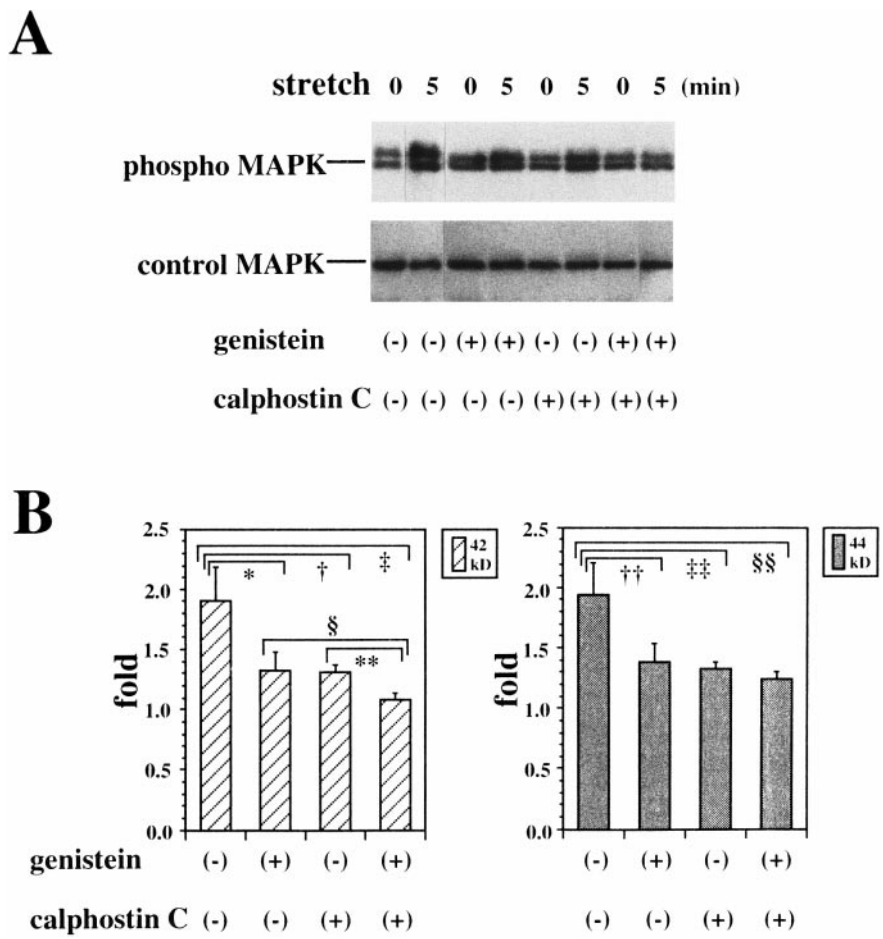

FIG. 4. Effects of tyrosine kinase inhibitor genistein and PKC inhibitor calphostin C on phosphorylation of p44/p42MAPK in cardiac myocytes subjected to pulsatile mechanical stretch. Serum-starved cardiac myocytes were pretreated with genistein, calphostin C, or both for 30 min and subjected to pulsatile mechanical stretch (60 cycles per min, 15% elongation) for 5 min. Cells were subsequently lysed with buffer A, and the cell lysates were subjected to Western analyses using a phospho-specific p44/p42MAPK (ERK) antibody (A, upper panel) or a control anti-p44/p42MAPK (ERK) antibody (A, lower panel). The antibody-antigen complexes were developed with chemiluminescence using alkalinephosphatase. The results shown are from one typical experiment. Panel B shows the fold induction of the phosphorylation of p44/p42MAPK at 5 min of stimulation as compared with that at 0 min. The results shown represent the (mean ± SD) from one typical experiment ($n = 3$). We performed at least three independent experiments. (p42MAPK, $*p < 0.01$, $\dagger p < 0.005$, $\ddagger p < 0.005$, $\S p < 0.05$, and $**p < 0.001$ versus control; p44MAPK, $\dagger\dagger p < 0.005$, $\ddagger\ddagger p < 0.001$, and $\S\S p < 0.0005$ versus control).

DISCUSSION

In the present study, we demonstrated that pulsatile mechanical stretch activated all three MAPK family members as well as $p125^{FAK}$. We also demonstrated that the pulsatile stretch-induced activation of ERKs was at least partly mediated by VEGF and TGF-β, and was also at least partly dependent on tyrosine kinases as well as PKC.

As for the effects of a single mechanical stretch on cardiac myocytes, up to now a lot of information including the intracellular signaling has been accumulated. It was reported that single mechanical stretch activated multiple second messenger pathways in cardiac myocytes including tyrosine kinases, RAS/MAPK pathway, PKC, phospholipase C and D, leading to c-*fos* induction (18). It was also reported that the single mechanical stretch-induced activation of intracellular signaling in cardiac myocytes was mediated by autocrine release of some humoral factor such as angiotensin II or endothelin (19, 4). In the present study, we have shown that pulsatile stretch-induced activation of ERKs was at least partially (but apparently mainly) mediated by VEGF, which was shown to be induced by TGF-β (9), but not by angiotensin II nor endothelin. This strongly suggests that different mechanisms were involved in the activation of intracellular signaling between in cardiac myocytes subjected to a single stretch and in those subjected to pulsatile stretch. We think that pulsatile stretch (such as 60 cycles per min) may cause relative myocardial hypoxia which in turn induces VEGF to adapt to the hypoxic condition.

Whereas single stretch is thought to be not sufficient for the induction of relative hypoxic condition, rather it may mainly cause hypertrophic responses in cardiac myocytes. For the effects of pulsatile stretch on other cell types, it was reported that pulsatile stretch stimulated expression and secretion of TGF-β by glomerular mesangial cells *in vitro* (20, 21), which is consistent with the present data in cardiac myocytes. Because TGF-β is a potent inducer of VEGF (22, 23), the results of the present study strongly suggest that the humoral factor directly triggering the pulsatile stretch-induced intracellular signaling in cardiac myocytes is VEGF. The present data are also supported by our previous findings that VEGF activates MAPK pathway as well as $p125^{FAK}$ in cardiac myocytes (24, 25). Adhesive interactions between cells and the extracellular matrix (ECM) play a pivotal role in the cell morphology, motility, and growth as well as gene expression in a variety of cell types. It is known that integrin-mediated cell adhesion causes increased tyrosine phosphorylation of $p125^{FAK}$ to link the ECM with the actin cytoskeleton and also transduce extracellular signals to the cell interior (26). Therefore, it is unclear whether the pulsatile stretch-induced activation of $p125^{FAK}$ revealed in the present study was due to the direct mechanical effect on cardiac myocytes or the effect of VEGF or both.

It was reported that single stretch-induced activation of intracellular signaling and c-*fos* induction in cardiac myocytes was primarily dependent on PKC (27, 18). As for pulsatile stretch in glomerular mesangial cells, there have been two papers reporting somewhat contradictory results. One paper showed that PKC inhibitor blocked the activation of S6 kinase (28), whereas the other paper showed that tyrosine kinase inhibitor but not PKC inhibitor blocked the induction of TGF-β expression (21). Because known VEGF receptors (Flt-1 and KDR/Flk-1) are tyrosine kinases, the effects of VEGF are thought to be tyrosine kinase dependent. On the other hand, cardiac responses to the pure mechanical stretch as shown in the single stretch studies are thought to be at least partly dependent on PKC. Therefore, the results of the present study that pulsatile stretch-induced activation of intracellular signaling was at least partly dependent on tyrosine kinases as well as PKC seem to be reasonable. However, we can not exclude the possibility that other humoral factors are involved in the pulsatile stretch-induced intracellular signaling in cardiac myocytes. The activated intracellular signaling pathways will cross talk with each other and converge to regulate expression of various genes to adapt to this external stress.

ACKNOWLEDGMENTS

This work was supported by a grant for cardiomyopathy from the Ministry of Health and Welfare, Japan, a grant for scientific research from the Ministry of Education, Science and Culture, Japan, a grant from Sankyo Foundation of Life Science, Japan Heart Foundation/ Pfizer Pharmaceuticals Grant for Research on Coronary Artery Disease, and a grant from Study Group of Molecular Cardiology. We thank Dr. Hisataka Sabe (Department of Molecular Biology, Osaka BioScience Institute, Osaka, Japan) for anti-$p125^{FAK}$ polyclonal antibody. We also thank Kaori Takahashi for excellent technical assistance.

REFERENCES

1. Takahashi, T., Schunkert, H., Isoyama, S., Wei, J. Y., Nadal-Ginard, B., Grossman, W., and Izumo, S. (1992) *J. Clin. Invest.* **89,** 939–946.
2. Sadoshima, J., Xu, Y., Slayter, H. S., and Izumo, S. (1993) *Cell* **75,** 977–984.
3. Everett, A. D., Tufro-McReddie, A., Fisher, A., and Gomez, R. A. (1994) *Hypertension* **23,** 587–592.
4. Yamazaki, T., Komuro, I., Kudoh, S., Zou, Y., Shiojima, I., Hiroi, Y., Mizuno, T., Maemura, K., Kurihara, H., Aikawa, R., Takano, H., and Yazaki, Y. (1996) *J. Biol. Chem.* **271,** 3221–3228.
5. Hashimoto, E., Ogita, T., Nakaoka, T., Matsuoka, R., Takao, A., and Kira, Y. (1994) *Am. J. Physiol.* **267,** H1948–H1954.
6. Minchenko, A., Bauer, T., Salceda, S., and Caro, J. (1994) *Lab. Invest.* **71,** 374–379.
7. Li, J., Hampton, T., Morgan, J. P., and Simons, M. (1997) *J. Clin. Invest.* **100,** 18–24.
8. Seko, Y., Imai, Y., Suzuki, S., Kamijukkoku, S., Hayasaki, K., Sakomura, Y., Tobe, K., Kadowaki, T., Maekawa, H., Takahashi, N., and Yazaki, Y. (1997) *Clin. Sci.* **92,** 453–454.
9. Seko, Y., Seko, Y., Takahashi, N., Shibuya, M., and Yazaki, Y. (1999) *Biochem. Biophys. Res. Commun.* **254,** 462–465.
10. Seko, Y., Tobe, K., Ueki, K., Kadowaki, T., and Yazaki, Y. (1996) *Circ. Res.* **78,** 82–90.
11. Seko, Y., Tobe, K., Takahashi, N., Kaburagi, Y., Kadowaki, T., and Yazaki, Y. (1996) *Biochem. Biophys. Res. Commun.* **226,** 530–535.
12. Seko, Y., Takahashi, N., Tobe, K., Kadowaki, T., and Yazaki, Y. (1997) *Biochem. Biophys. Res. Commun.* **239,** 840–844.
13. Sadoshima, J., Xu, Y., Slayter, H. S., and Izumo, S. (1993) *Cell* **75,** 977–984.
14. Everett, A. D., Tufro-McReddie, A., Fisher, A., and Gomez, R. A. (1994) *Hypertension* **23,** 587–592.
15. Yang, Z., Noll, G., and Luscher, T. F. (1993) *Circulation* **88,** 832–836.
16. Tobe, K., Sabe, H., Yamamoto, T., Yamauchi, T., Asai, S., Kaburagi, Y., Tamemoto, H., Ueki, K., Kimura, H., Akanuma, Y., Yazaki, Y., Hanafusa, H., and Kadowaki, T. (1996) *Mol. Cell. Biol.* **16,** 4765–4772.
17. Kobayashi, E., Nakano, H., Morimoto, M., and Tamaoki, T. (1989) *Biochem. Biophys. Res. Commun.* **159,** 548–553.
18. Sadoshima, J., and Izumo, S. (1993) *EMBO J.* **12,** 1681–1692.
19. Sadoshima, J., Xu, Y., Slayter, H. S., and Izumo, S. (1993) *Cell* **75,** 977–984.
20. Yasuda, T., Kondo, S., Homma, T., and Harris, R. C. (1996) *J. Clin. Invest.* **98,** 1991–2000.
21. Hirakata, M., Kaname, S., Chung, U.-G., Joki, N., Hori, Y., Noda, M., Takuwa, Y., Okazaki, T., Fujita, T., Katoh, T., Kurokawa, K. (1997) *Kidney Int.* **51,** 1028–1036.
22. Brogi, E., Wu, T., Namiki, A., and Isner, J. M. (1994) *Circulation* **90,** 649–652.
23. Pertovaara, L., Kaipainen, A., Mustonen, T., Orpana, A., Ferrara, N., Saksela, O., and Alitalo, K. (1994) *J. Biol. Chem.* **269,** 6271–6274.

24. Seko, Y., Takahashi, N., Tobe, K., Ueki, K., Kadowaki, T., and Yazaki, Y. (1998) *J. Cell Physiol.* **175,** 239–246.
25. Takahashi, N., Seko, Y., Noiri, E., Tobe, K., Kadowaki, T., and Yazaki, Y. *Circ. Res.* (in press).
26. Juliano, R. L., and Haskill, S. (1993) *J. Cell Biol.* **120,** 577–585.
27. Yamazaki, T., Tobe, K., Hoh, E., Maemura, K., Kaida, T., Komuro, I., Tamemoto, H., Kadowaki, T., Nagai, R., and Yazaki, Y. (1993) *J. Biol. Chem.* **268,** 12069–12076.
28. Homma, T., Akai, Y., Burns, K. D., and Harris, R. C. (1992) *J. Biol. Chem.* **267,** 23129–23135.