Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 329 (2005) 18–24
BBRC
www.elsevier.com/locate/ybbrc

Leptin induces hypertrophy via p38 mitogen-activated protein kinase in rat vascular smooth muscle cells

Hye-Jin Shin[a,b], Jaewon Oh[a,b], Seok Min Kang[b], Jong Ho Lee[a], Min-Jeong Shin[b], Ki-Chul Hwang[b], Yangsoo Jang[a,b,c,*], Ji Hyung Chung[a,b,d,*]

[a] *Yonsei Research Institute of Aging Science, Yonsei University, Seoul 120-749, Republic of Korea*
[b] *Cardiovascular Research Institute, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea*
[c] *Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea*
[d] *Molecular Aging Research Center, Yonsei University, Seoul 120-749, Republic of Korea*

Received 29 November 2004
Available online 1 February 2005

Abstract

The hypertrophy of vascular smooth muscle cells (VSMCs) is critical in vascular remodeling associated with hypertension, atherosclerosis, and restenosis. Recently, leptin has appeared to play a pivotal role in vascular remodeling. However, the mechanism by which leptin induces hypertrophy in vascular smooth muscle cells is still unknown. We studied the role of leptin as a potential hypertrophic factor in rat VSMCs. In the present study, leptin significantly increased [^{3}H]leucine incorporation and the total protein/DNA ratio in VSMCs. The maximal hypertrophic effect was at 100 ng/ml of leptin. Leptin induced phosphorylation and activation of p38 mitogen-activated protein (p38 MAP) kinase and of signal transducers and activators of transcription 3 in a concentration- and time-dependent manner. A p38 MAP kinase inhibitor SB203580 significantly inhibited leptin-induced hypertrophy, AG490 (a JAK2 inhibitor) partially inhibited it, and other MAP kinase inhibitors, PD98059 (an ERK inhibitor) and SP600125 (a JNK inhibitor), had no effect. These results indicate that leptin directly stimulates cellular hypertrophy via p38 MAP kinase in rat VSMCs.

Keywords: Hypertrophy; Leptin; p38 MAP kinase; Vascular smooth muscle cells; SB203580

Leptin, the 16-kDa peptide released from fat cells and other tissues, acts on the hypothalamic leptin receptors (Ob-R) to decrease food intake and increase energy expenditure [1]. Leptin was initially thought to act mainly to regulate obesity or body weight. However, recent studies have demonstrated the effects of leptin in the regulation of inflammation [2], blood pressure homeostasis, and cardiovascular disease [3]. Leptin receptors have a widespread tissue distribution, including kidney, pancreas, lung, and heart [4–6]. Leptin has angiogenic effects through proliferative effects on vascular endothelial cells [7] and stimulates proliferation and migration in smooth muscle cells through activation of mitogen-activated protein (MAP) kinases [8]. This evidence suggests that leptin may also have a wide-ranging influence on the metabolism and signaling of vascular cells and possibly also on the cardiovascular system. Recent clinical evidence has shown increased levels of plasma leptin in patients with heart failure [9,10]. Furthermore, leptin also appears to be an important factor in vascular remodeling [11], arterial restenosis [12], and cardiac hypertrophy [13].

The hypertrophy of vascular smooth muscle cells (VSMCs), which is characterized by increased protein synthesis, is critical in vascular remodeling associated with hypertension, atherosclerosis, and restenosis. Several circulating factors and environmental stimuli are believed to be responsible for VSMC hypertrophy in the vessel wall. Recent studies have focused on the

* Corresponding authors. Fax: +82 2 364 9605.
E-mail address: jhchung@yonsei.ac.kr (J.H. Chung).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.12.195

signaling events that influence this cellular hypertrophy in VSMCs in vitro. Angiotensin II stimulates NAD(P)H oxidases to increase the production of reactive oxygen species, which, in turn, mediates p38 MAPK and Akt activation, both of which are required for hypertrophy in VSMCs [14]. Norepinephrine also activates a MAP kinase pathway including p38 MAP kinase, leading to cellular hypertrophy in VSMCs [15].

Leptin activates a variety of signaling pathways in a number of cell lines [16]. The signal Janus kinase (JAK)/signal transducer and activator of transcription activation (STAT) pathway mainly transmits leptin signaling; however, recent studies indicate that leptin can also use other signaling cascades such as extracellular signal-regulated kinase (ERK1/2) [17], c-Jun terminal/stress-activated protein kinase (JNK) [18], and p38 MAP kinase [19] of MAP kinase family members in a variety of cell types. Interestingly, recent data using animal models and cultured cardiomyocytes indicate that leptin is directly linked with cardiac hypertrophy [20,21]. However, it is still unclear whether leptin directly induces VSMC hypertrophy and which mechanisms are involved in hypertrophic responses to leptin stimuli in vitro. Because many hypertrophy factors, including circulating factors and mechanical stresses, share similar signaling events such as MAP kinase activation, we hypothesized that the induction of leptin-activated MAP kinases would be important for VSMC hypertrophy. In the present study, we examined the hypertrophic effect of leptin on cultured rat VSMCs and sought to identify potential mechanisms underlying this effect.

Materials and methods

Materials. Leptin was purchased from BioSource (Camarillo, CA). SB203580 (a p38 MAP kinase inhibitor) and PD98059 (an ERK inhibitor) were obtained from A.G. Scientific (San Diego, CA). JAK2 inhibitor AG490 (Tyrphostin B42) and JNK inhibitor SP600125 were obtained from Calbiochem (La Jolla, CA). Phospho-specific p38 MAP kinase antibody, p38 MAP kinase antibody, MAP kinase kinase (MKK) 6 antibody, and STAT3 antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Phospho-MKK3/6 antibody, MKK3 antibody, and phospho-specific STAT3 (Tyr705) antibody were purchased from Cell Signaling (Beverly, MA). Horseradish peroxidase-conjugated secondary antibodies and an enhanced chemiluminescence (ECL) Western blotting detection system were obtained from Santa Cruz Biotechnology and Amersham Biosciences (Uppsala, Sweden), respectively. Other materials and chemicals were obtained from commercial sources.

Cell culture. VSMCs were isolated as previously described [22]. Briefly, the aorta was enzymatically isolated from the thoracic aortas from 8-week-old Sprague–Dawley rats. The aorta was transferred to a plastic tube containing 5 ml of the enzyme dissociation mixture and incubated for 2 h at 37 °C. The suspension was centrifuged at 1000 rpm for 10 min and the pellet was resuspended in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS). The cells were maintained in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin in 75-cm^2 flasks at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. All experiments were conducted using VSMCs between passage numbers 7 and 15 that were growth-arrested by incubation for 48 h in DMEM containing 0.5% FBS.

SDS–PAGE and Western blot analysis. Growth-arrested VSMCs were treated with leptin in the presence or absence of appropriate inhibitors for the indicated time periods at 37 °C. The medium was aspirated, and the cells were rinsed with cold phosphate-buffered saline (PBS) and immediately scraped into 1.5-ml tubes at −80 °C. Harvested cells were solubilized in a lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, and 1 μg/ml leupeptin) and centrifuged at 12,000*g* for 20 min at 4 °C. The protein concentrations of the supernatants were determined using a Bradford protein assay kit (Bio-Rad, Hercules, CA). Cell lysates containing equal amounts of protein were subjected to SDS–polyacrylamide gel electrophoresis, and the proteins were transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA). After blocking in 10 mM Tris–HCl buffer (pH 8.0) containing 150 mM sodium chloride, 0.1% Tween 20, and 5% (w/v) nonfat dry milk, the membrane was treated with appropriate primary antibodies, followed by incubation with appropriate horseradish peroxidase-conjugated secondary antibodies. The antigen–antibody bands were detected using an enhanced chemiluminescence reagent kit (Amersham Bioscience, Uppsala, Sweden) and quantified by densitometry.

p38 MAP kinase activity assay. A p38 MAP kinase activity assay was carried out following the instructions from the commercial assay kit (Cell Signaling, Beverly, MA). Briefly, growth-arrested VSMCs were treated with 100 ng/ml leptin for various times as indicated. Cells were harvested with lysis buffer supplied by the manufacturer and centrifuged at 12,000*g* for 10 min at 4 °C. The cell lysates were incubated overnight at 4 °C with 20 μl of an immobilized dual phospho-specific p38 MAP kinase (Thr180/Tyr182) monoclonal antibody. The samples were then washed twice with lysis buffer and kinase buffer. The kinase reaction was performed at 30 °C for 10 min in the presence of 200 μM ATP and 2 μg of an activating transcription factor (ATF)-2 fusion protein. The reaction was then terminated with 25 μl of SDS loading buffer. Phosphorylation of ATF-2 at Thr71 was measured by Western blot analysis using a specific antibody against phospho-ATF-2 (Thr71) and LumiGLO system supplied by the manufacturer.

Total protein/DNA ratio. Cells plated in 60-cm^2 plates were growth-arrested as described above and then incubated in leptin for 24 h. Cells were washed in ice-cold PBS and lysed in 0.3 M NaOH. Total protein content was determined colorimetrically using Bradford protein assay reagent (Bio-Rad, Hercules, CA). For the quantitative measurement of DNA, cells were lysed by adding SDS and proteinase K, and extraction of DNA was performed with phenol. The absorbance of the purified DNA was measured at 260 nm. Serial concentrations of calf thymus DNA and BSA were used for the calibration curves.

[^{3}H]Leucine incorporation assay. [^{3}H]Leucine incorporation was measured as described previously [23]. VSMCs were grown in 24-well plates and made quiescent in serum-free medium for 48 h. VSMCs were then treated with leptin for 24 h after pretreatment with or without inhibitors for 1 h. The stimulated cells were pulsed with 2 μCi/ml [^{3}H]leucine during the last 6 h before harvest. After being washed with ice-cold PBS, cells were treated with 5% trichloroacetic acid for 30 min and then washed twice with ice-cold PBS. Finally, cells were solubilized in 300 μl of 1 N NaOH for 30 min. After neutralization with 0.5 N HCl, an aliquot was taken to determine the incorporated radioactivity by liquid scintillation counting. Radioactivity was measured in a liquid scintillation counter (Beckman LS 3801, Fullerton, CA).

Statistical analysis. Data are expressed as means ± SE. Statistical comparisons were performed by one-way analysis of variance and Student's *t* test. Differences were considered significant at $P < 0.05$.

Results

Leptin-induced hypertrophy in rat VSMCs

To examine the hypertrophic responses to leptin, first the total DNA and protein contents were determined in rat VSMCs with leptin treatment. Rat VSMCs were treated with different concentrations (1–500 ng/ml) of leptin for 24 h, and cell hypertrophy was assessed by calculating the ratio of total protein to DNA content. We observed that leptin increased protein/DNA content in a concentration-dependent manner (Fig. 1A). After 24 h of leptin treatment, significantly augmented protein/DNA content occurred at concentrations up to 100 ng/ml, indicating that the VSMCs were synthesizing more protein than DNA, suggesting cellular hypertrophy. A similar result was obtained using the [^{3}H]leucine incorporation assay (Fig. 1B). After 24 h of stimulation, 100 ng/ml leptin significantly increased the [^{3}H]leucine incorporation to 144%, based on the no treatment option ($P < 0.05$). No significant changes were observed at higher leptin concentrations (~500 ng/ml) (Fig. 1). Since the maximal hypertrophy appeared to be afforded by treatment with 100 ng/ml leptin, we used this concentration in the following experiments. This concentration of leptin occurred in the absence of any increased VSMC deaths (data not shown).

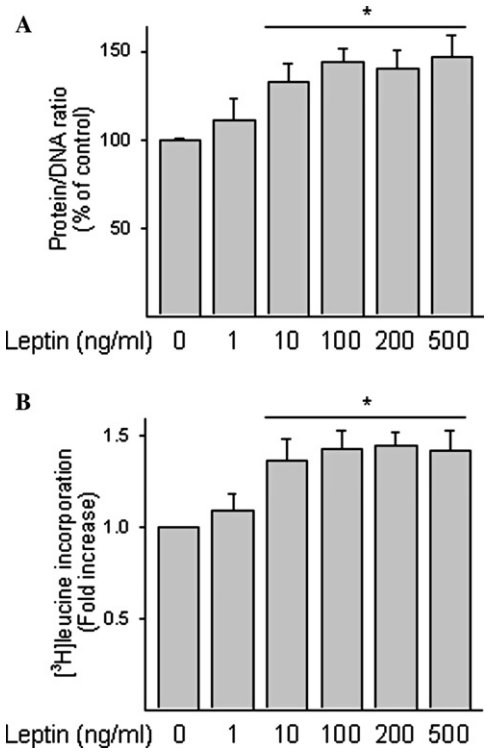

Fig. 1. Leptin-induced cellular hypertrophy in rat VSMCs. (A) Quiescent rat VAMCs were incubated with different concentrations of leptin for 24 h. Total protein and DNA contents of VSMCs were measured using the Bradford protein assay reagent and the phenol extraction method, respectively. (B) Quiescent rat VSMCs were incubated with different concentrations of leptin in 24-well plates for 24 h. Cells were treated with 2 μCi/ml [^{3}H]leucine during the last 6 h before harvest. At the end of labeling, cells were washed with ice-cold PBS and treated with 5% trichloroacetic acid for 30 min. Cells were solubilized in 300 μl of 1 N NaOH for 30 min. After neutralization with 0.5 N HCl, an aliquot was taken to determine the incorporated radioactivity by liquid scintillation counting. Results are indicated as means ± SE from three separate experiments. *$P < 0.05$ compared to the control group.

Leptin-induced hypertrophy is inhibited by p38 MAP kinase inhibitor

Although the signal pathways regulated by leptin are diverse [16,24], the selective pathways through which leptin induces cellular hypertrophy in VSMCs are not yet defined. In relation to the possible participation of protein signaling in VSMCs hypertrophy induced by leptin, the effects of several inhibitors were studied. Since activation of the MAP kinase signal pathways has been demonstrated both in vitro [18,25,26] and in vivo [27,28] in different cell types, we investigated the role of MAP kinases as potential mediators of the hypertrophic effect of leptin. Interestingly, treatment with the p38 MAP kinase inhibitor SB203580 significantly inhibited the effect of leptin on hypertrophy of VSMCs (Fig. 2). Neither PD98059 (an ERK inhibitor) nor SP600125 (a JNK inhibitor) significantly affected the hypertrophy of VSMCs. We next examined whether

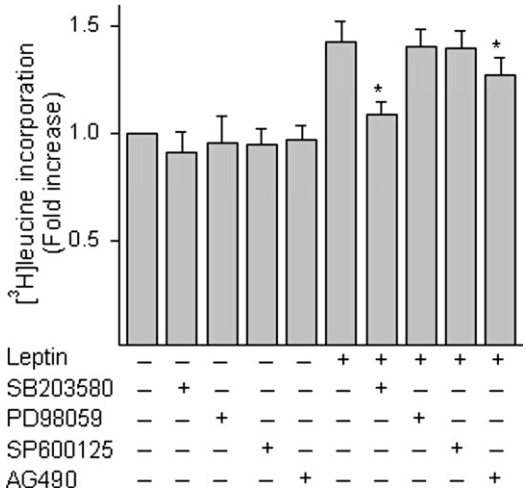

Fig. 2. Effect of inhibitors on leptin-induced hypertrophy in rat VSMCs. Rat VSMCs were seeded in 24-well plates and cultured in serum-free DMEM. Cells were pretreated for 1 h with SB203580 (25 μM), PD98059 (25 μM), SP600125 (25 μM), or AG490 (25 μM). Then cells were stimulated with leptin (100 ng/ml) for 24 h with pulsed addition of [^{3}H]leucine for the final 6 h. At the end of labeling, cells were washed with ice-cold PBS and treated with 5% trichloroacetic acid for 30 min. Cells were solubilized in 300 μl of 1 N NaOH for 30 min. After neutralization with 0.5 N HCl, an aliquot was taken to determine the incorporated radioactivity by liquid scintillation counting. Results are indicated as means ± SE from three different experiments. *$P < 0.05$ compared to leptin alone.

JAK/STAT signals are also involved in leptin-induced hypertrophy using a JAK2 inhibitor, AG490. It has been known that the JAK/STAT pathway plays a critical role in the effects of many cytokines [29] and mainly transmits signals in response to leptin in the pituitary and some cell types [30–33]. As shown in Fig. 2, AG490 had a partial inhibitory effect on leucine incorporation by leptin stimulation. A similar result was obtained using the protein/DNA content ratio assay (data not shown). Taken together, these results suggest that leptin directly stimulates cellular hypertrophy via p38 MAP kinase in rat VSMCs.

Leptin activates p38 MAP kinase pathway

To further confirm whether the hypertrophy mechanism of leptin was involved in p38 MAP kinase signaling, we primarily examined the effect of leptin on the activation of p38 MAP kinase. VSMCs were treated with 100 ng/ml leptin for various times, and then p38 MAP kinase was determined by Western blot with phospho-specific p38 MAP kinase antibody. We found that leptin strongly induced phosphorylation of p38 MAP kinase in VSMCs. Phosphorylation of p38 MAP kinase gradually increased for 60 min (Fig. 3A). The protein levels of total p38 MAP kinase were unchanged throughout the time course. In addition to analysis of phosphorylation of p38 MAP kinase, we next examined whether p38 MAP kinase phosphorylation by leptin was compatible with its activity. After leptin stimulation under the above conditions, p38 MAP kinase activity was analyzed by immunoprecipitation with an immobilized p38 MAP kinase antibody followed by in vitro kinase assay with an ATF-2 fusion protein as a substrate of p38 MAP kinase. As shown in Fig. 3B, leptin induced p38 MAP kinase activity, as demonstrated by the increased phosphorylation of ATF-2. Its activity was gradually increased with a time course of 60 min, consistent with the results of the Western blot data. We also investigated the effects of different concentrations of leptin on p38 MAP kinase activity in VSMCs. Leptin stimulated p38 MAP kinase phosphorylation in a concentration-dependent manner without changes in p38 MAP kinase protein levels (Fig. 4A). The p38 MAP kinase is activated by an upstream regulator, MAP kinase kinase 3/6 (MKK3/6) [34]. To determine which of these kinases is responsible for the activation of p38 MAP kinase in leptin-stimulated VSMCs, their phosphorylation was investigated. The MKK3/6 phosphorylation was increased with the same trend as that of p38 MAP kinase in a concentration-dependent manner (Fig. 4B). The protein levels of MKK3 or MKK6 were not affected by any stimulation. These data suggest that leptin can activate p38 MAP kinase via an MKK3/6-dependent pathway following cellular hypertrophy in VSMCs.

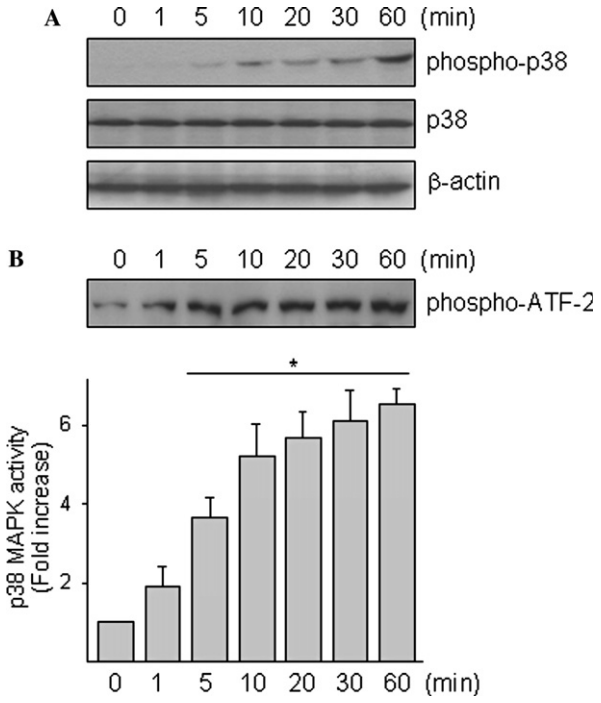

Fig. 3. Phosphorylation and activation of p38 MAP kinase by leptin stimulation in rat VSMCs. (A) Quiescent rat VSMCs were treated with 100 ng/ml leptin for the indicated time periods. Equal amounts of protein were separated by SDS–PAGE gel and analyzed by Western blotting. Phosphorylation of p38 MAP kinase was detected using a phospho-specific p38 MAP kinase antibody. Blots are representative of three experiments, all with similar results. (B) Quiescent rat VSMCs were treated with 100 ng/ml leptin for various times as indicated. Cells were lysed and the same amount of extracted proteins was immunoprecipitated with a phospho-specific p38 MAP kinase antibody. Samples were incubated with an ATF-2 protein in the presence of ATP. Phosphorylation of ATF-2 was measured by Western blot analysis using the phospho-ATF-2 (Thr71) antibody. Each signal was quantified by scanning densitometry and the graph shows the levels of each activity as relative value. Results are indicated as means ± SE from three different experiments. $*P < 0.05$ compared to the control group.

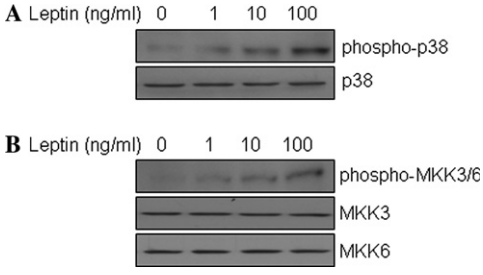

Fig. 4. Effect of leptin on phosphorylation of p38 MAP kinase and MKK3/6 in rat VSMCs. Rat VSMCs were treated with different concentrations of leptin for 1 h. Equal amounts of protein were separated by SDS–PAGE gel and analyzed by Western blotting. Phosphorylation of p38 MAP kinase (A) and MKK3/6 (B) was detected using a phospho-p38 MAP kinase antibody and a phospho-MKK3/6 antibody, respectively. Results shown are representative of at least three independent experiments with similar results.

Discussion

The present study showed that leptin directly stimulates cellular hypertrophy via p38 MAP kinase in rat VSMCs. Leptin has been demonstrated to induce proliferation, angiogenesis, or functional activation of hematopoietic cells, pancreatic cells, and vascular cells [8,26,35,36]. Recent clinical evidence has shown that increased plasma leptin levels are correlated with cardiovascular disorders and risk of cardiovascular diseases [3,27]. Leptin may also play an important role in regulating vascular tone and neovascularization because its functional receptors (Ob-R) are expressed in the vascular cells [37]. Also, there are some studies of the effect of leptin on the proliferation of VSMCs [8,11]. However, whether leptin directly induces cellular hypertrophy in VSMCs remained unclear. Therefore, in the present study, we examined whether leptin can induce cellular hypertrophy of VSMCs and which signal pathways are involved in this event. First we determined the effect of leptin on the cellular hypertrophy of rat VSMCs using total protein/DNA ratio and [^{3}H]leucine incorporation assay. Leptin significantly induced cellular hypertrophy, which peaked at a concentration of 100 ng/ml (Fig. 1). Also, the relationship between cellular hypertrophy and MAP kinases was determined. In Fig. 2, the p38 MAP kinase inhibitor SB203580 caused almost complete inhibition of leptin-induced hypertrophy. Moreover, leptin stimulation enhanced the phosphorylation and activity of p38 MAP kinase in VSMCs. These results indicate that the p38 MAP kinase pathway regulates leptin-induced cellular hypertrophy of VSMCs.

It has been known that p38 MAP kinase is activated by a diverse range of physical and chemical stresses, such as various cytokines, hypoxia/ischemia, oxidative stress, and UV irradiation [38]. Recently, a few studies have addressed the role of p38 MAP kinase in leptin signaling in different cell types [19,39]. Also, it has previously been shown that p38 MAP kinase mediate angiotensin II-induced [40] or norepinephrine-induced [15] VSMC hypertrophy. However, little is known about the hypertrophy mechanism of leptin in VSMCs. The present study is the first observation of leptin-stimulated hypertrophy through the p38 MAP kinase pathway. The MAP kinases are activated by phosphorylation by MAP kinase kinases (MKKs), which are very specific for downstream MAP kinases [41]. Our data showed that the phosphorylation of MKK3/6 had a tendency similar to that of p38 MAP kinase in concentration-dependent behavior. The activity of these kinases was consistent with leucine incorporation results shown in Figs. 1 and 2, indicating that the p38 MAP kinase cascade mainly contributes to the process of cellular hypertrophy by leptin.

Leptin has several other effects mediated by the activation of the JAK/STAT pathway in different cell types. Also, current data demonstrated that the key intracellular components of the leptin signaling pathway are the kinase JAK2 and the STAT3 [31,32,42]. In our study, leptin also induced phosphorylation of STAT3 (Tyr705) in a concentration- and time-dependent manner (Fig. 5). In addition, we found that leptin-induced STAT3 phosphorylation was inhibited by AG490, indicating mediation of STAT3 activation by JAK2. AG490 treatment also inhibited leptin-induced cell hypertrophy, but the inhibition was not comparable to the results of SB203580 treatment (Fig. 2). This result indicates that JAK2/STAT3, even if in part, might be involved in the regulation of leptin-induced hypertrophy in VSMCs. One possibility is that leptin-induced hypertrophy might be regulated by the simultaneous activation of two distinct pathways, p38 MAP kinase and JAK2/STAT3. Another possibility is that leptin induces VSMC hypertrophy exclusively through the p38 MAP kinase pathway, and activation of this pathway requires JAK2/STAT3 and other intracellular signals. These mechanisms require further studies.

Recently, it has been reported that leptin also correlates with other markers of metabolic syndromes, independent of obesity, and elevated plasma leptin levels may be an independent risk factor for the cardiovascular diseases [3]. According to the West of Scotland Coronary Prevention Study (WOSCOPS), plasma leptin levels are higher in coronary heart disease (CHD) patients, and leptin is an independent risk factor for CHD [43]. Recent clinical data also showed that leptin is associated with impaired arterial distensibility [44], coronary vasoreactivity [45], and coronary atherosclerosis [46]. Piatti et al. [47], in particular, reported that

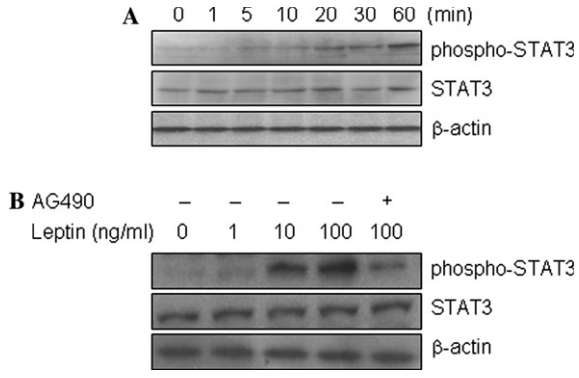

Fig. 5. Phosphorylation of STAT3 by leptin stimulation in rat VSMCs. (A) Quiescent rat VSMCs were treated with 100 ng/ml leptin for the indicated time periods. Equal amounts of protein were separated by SDS–PAGE gel and analyzed by Western blotting using phospho-specific STAT3 (Tyr705) antibody. Blots are representative of three experiments, all with similar results. (B) Rat VSMCs were treated with different concentrations of leptin for 1 h. Equal amounts of protein were separated by SDS–PAGE gel and analyzed by Western blotting. AG490 (a JAK2 inhibitor) of 25 μM was pretreated for 1 h before leptin stimulation. Results shown are representative of at least three independent experiments with similar results.

leptin level was higher in patients with restenosis after coronary stenting. Recent in vivo data showed that leptin promoted vascular remodeling and neointimal growth [11,12]. It has been also reported that leptin directly induces smooth muscle cell proliferation and migration in vitro [8]. However, it has not yet been determined whether leptin directly stimulates smooth muscle cell hypertrophy in vitro.

In conclusion, we have shown the hypertrophic effect of leptin and the relevant signal transduction mechanism in rat VSMCs, in vitro. In the present study, we have shown that in VSMCs, leptin induced cellular hypertrophy via the activation of the p38 MAP kinase pathway, indicating the potential role of leptin in the development of the cardiovascular system and the vascular remodeling of cardiovascular diseases.

Acknowledgments

This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ10-PG6-AG01-0010) (Molecular Aging Research Center).

References

[1] E. Jequier, Leptin signaling, adiposity, and energy balance, Ann. N.Y. Acad. Sci. 967 (2002) 379–388.
[2] A. La Cava, C. Alviggi, G. Matarese, Unraveling the multiple roles of leptin in inflammation and autoimmunity, J. Mol. Med. 82 (2004) 4–11.
[3] J. Ren, Leptin and hyperleptinemia—from friend to foe for cardiovascular function, J. Endocrinol. 181 (2004) 1–10.
[4] N. Hoggard, J.G. Mercer, D.V. Rayner, K. Moar, P. Trayhurn, L.M. Williams, Localization of leptin receptor mRNA splice variants in murine peripheral tissues by RT-PCR and in situ hybridization, Biochem. Biophys. Res. Commun. 232 (1997) 383–387.
[5] T.J. Kieffer, R.S. Heller, J.F. Habener, Leptin receptors expressed on pancreatic beta-cells, Biochem. Biophys. Res. Commun. 224 (1996) 522–527.
[6] J. Lin, C.R. Barb, R.L. Matteri, R.R. Kraeling, X. Chen, R.J. Meinersmann, G.B. Rampacek, Long form leptin receptor mRNA expression in the brain, pituitary, and other tissues in the pig, Domestic Anim. Endocrinol. 19 (2000) 53–61.
[7] M. Artwohl, M. Roden, T. Holzenbein, A. Freudenthaler, W. Waldhausl, S.M. Baumgartner-Parzer, Modulation by leptin of proliferation and apoptosis in vascular endothelial cells, Int. J. Obes. Relat. Metab. Disord. 26 (2002) 577–580.
[8] A. Oda, T. Taniguchi, M. Yokoyama, Leptin stimulates rat aortic smooth muscle cell proliferation and migration, Kobe J. Med. Sci. 47 (2001) 141–150.
[9] P.C. Schulze, J. Kratzsch, A. Linke, N. Schoene, V. Adams, S. Gielen, S. Erbs, S. Moebius-Winkler, G. Schuler, Elevated serum levels of leptin and soluble leptin receptor in patients with advanced chronic heart failure, Eur. J. Heart Fail. 5 (2003) 33–40.
[10] R. Wolk, B.C. Johnson, V.K. Somers, Leptin and the ventilatory response to exercise in heart failure, J. Am. Coll. Cardiol. 42 (2003) 1644–1649.
[11] K. Schafer, M. Halle, C. Goeschen, C. Dellas, M. Pynn, D.J. Loskutoff, S. Konstantinides, Leptin promotes vascular remodeling and neointimal growth in mice, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 112–117.
[12] K. Stephenson, J. Tunstead, A. Tsai, R. Gordon, S. Henderson, H.M. Dansky, Neointimal formation after endovascular arterial injury is markedly attenuated in db/db mice, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 2027–2033.
[13] M. Pladevall, K. Williams, H. Guyer, J. Sadurni, C. Falces, A. Ribes, C. Pare, C. Brotons, R. Gabriel, M. Serrano-Rios, S. Haffner, The association between leptin and left ventricular hypertrophy: A population-based cross-sectional study, J. Hypertens. 21 (2003) 1467–1473.
[14] Y. Taniyama, M. Ushio-Fukai, H. Hitomi, P. Rocic, M.J. Kingsley, C. Pfahnl, D.S. Weber, R.W. Alexander, K.K. Griendling, Role of p38 MAPK and MAPKAPK-2 in angiotensin II-induced Akt activation in vascular smooth muscle cells, Am. J. Physiol. Cell Physiol. 287 (2004) C494–C499.
[15] S. Kalyankrishna, K.U. Malik, Norepinephrine-induced stimulation of p38 mitogen-activated protein kinase is mediated by arachidonic acid metabolites generated by activation of cytosolic phospholipase A(2) in vascular smooth muscle cells, J. Pharmacol. Exp. Ther. 304 (2003) 761–772.
[16] G. Sweeney, Leptin signaling, Cell Signal. 14 (2002) 655–663.
[17] C. Bjorbaek, R.M. Buchholz, S.M. Davis, S.H. Bates, D.D. Pierroz, H. Gu, B.G. Neel, M.G. Myers Jr., J.S. Flier, Divergent roles of SHP-2 in ERK activation by leptin receptors, J. Biol. Chem. 276 (2001) 4747–4755.
[18] A. Bouloumie, T. Marumo, M. Lafontan, R. Busse, Leptin induces oxidative stress in human endothelial cells, FASEB J. 13 (1999) 1231–1238.
[19] G.R. van den Brink, T. O'Toole, J.C. Hardwick, D.E. van den Boogaardt, H.H. Versteeg, S.J. van Deventer, M.P. Peppelenbosch, Leptin signaling in human peripheral blood mononuclear cells, activation of p38 and p42/44 mitogen-activated protein (MAP) kinase and p70 S6 kinase, Mol. Cell. Biol. Res. Commun. 4 (2000) 144–150.
[20] F.P. Xu, M.S. Chen, Y.Z. Wang, Q. Yi, S.B. Lin, A.F. Chen, J.D. Luo, Leptin induces hypertrophy via endothelin-1-reactive oxygen species pathway in cultured neonatal rat cardiomyocytes, Circulation 110 (2004) 1269–1275.
[21] L.A. Barouch, D.E. Berkowitz, R.W. Harrison, C.P. O'Donnell, J.M. Hare, Disruption of leptin signaling contributes to cardiac hypertrophy independently of body weight in mice, Circulation 108 (2003) 754–759.
[22] S. Gunther, R.W. Alexander, W.J. Atkinson, M.A. Gimbrone Jr., Functional angiotensin II receptors in cultured vascular smooth muscle cells, J. Cell Biol. 92 (1982) 289–298.
[23] Y. Zheng, H.J. Song, C.H. Kim, H.S. Kim, E.G. Kim, A. Sachinidis, H.Y. Ahn, Inhibitory effect of epigallocatechin 3-*O*-gallate on vascular smooth muscle cell hypertrophy induced by angiotensin II, J. Cardiovasc. Pharmacol. 43 (2004) 200–208.
[24] K. Hegyi, K. Fulop, K. Kovacs, S. Toth, A. Falus, Leptin-induced signal transduction pathways, Cell Biol. Int. 28 (2004) 159–169.
[25] A.S. Banks, S.M. Davis, S.H. Bates, M.G. Myers Jr., Activation of downstream signals by the long form of the leptin receptor, J. Biol. Chem. 275 (2000) 14563–14572.
[26] K. Tanabe, S. Okuya, Y. Tanizawa, A. Matsutani, Y. Oka, Leptin induces proliferation of pancreatic beta cell line MIN6 through activation of mitogen-activated protein kinase, Biochem. Biophys. Res. Commun. 241 (1997) 765–768.
[27] Y. Figenschau, G. Knutsen, S. Shahazeydi, O. Johansen, B. Sveinbjornsson, Human articular chondrocytes express functional leptin receptors, Biochem. Biophys. Res. Commun. 287 (2001) 190–197.

[28] T. Yamashita, T. Murakami, S. Otani, M. Kuwajima, K. Shima, Leptin receptor signal transduction: OBRa and OBRb of fa type, Biochem. Biophys. Res. Commun. 246 (1998) 752–759.
[29] J. Bromberg, J.E. Darnell Jr., The role of STATs in transcriptional control and their impact on cellular function, Oncogene 19 (2000) 2468–2473.
[30] I. Tsumanuma, L. Jin, S. Zhang, J.M. Bayliss, B.W. Scheithauer, R.V. Lloyd, Leptin signal transduction in the HP75 human pituitary cell line, Pituitary 3 (2000) 211–220.
[31] I. Goren, J. Pfeilschifter, S. Frank, Determination of leptin signaling pathways in human and murine keratinocytes, Biochem. Biophys. Res. Commun. 303 (2003) 1080–1085.
[32] M. Cauzac, D. Czuba, J. Girard, S. Hauguel-de Mouzon, Transduction of leptin growth signals in placental cells is independent of JAK-STAT activation, Placenta 24 (2003) 378–384.
[33] V. Sanchez-Margalet, C. Martin-Romero, Human leptin signaling in human peripheral blood mononuclear cells: Activation of the JAK-STAT pathway, Cell. Immunol. 211 (2001) 30–36.
[34] T. Obata, G.E. Brown, M.B. Yaffe, MAP kinase pathways activated by stress: The p38 MAPK pathway, Crit. Care Med. 28 (2000) N67–N77.
[35] T. Gainsford, T.A. Willson, D. Metcalf, E. Handman, C. McFarlane, A. Ng, N.A. Nicola, W.S. Alexander, D.J. Hilton, Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells, Proc. Natl. Acad. Sci. USA 93 (1996) 14564–14568.
[36] M.R. Sierra-Honigmann, A.K. Nath, C. Murakami, G. Garcia-Cardena, A. Papapetropoulos, W.C. Sessa, L.A. Madge, J.S. Schechner, M.B. Schwabb, P.J. Polverini, J.R. Flores-Riveros, Biological action of leptin as an angiogenic factor, Science 281 (1998) 1683–1686.
[37] S.M. Kang, H.M. Kwon, B.K. Hong, D. Kim, I.J. Kim, E.Y. Choi, Y. Jang, H.S. Kim, M.S. Kim, H.C. Kwon, Expression of leptin receptor (Ob-R) in human atherosclerotic lesions: Potential role in intimal neovascularization, Yonsei Med. J. 41 (2000) 68–75.
[38] M.L. Correia, W.G. Haynes, Leptin, obesity and cardiovascular disease, Curr. Opin. Nephrol. Hypertens. 13 (2004) 215–223.
[39] G. Sweeney, J. Keen, R. Somwar, D. Konrad, R. Garg, A. Klip, High leptin levels acutely inhibit insulin-stimulated glucose uptake without affecting glucose transporter 4 translocation in l6 rat skeletal muscle cells, Endocrinology 142 (2001) 4806–4812.
[40] M. Ushio-Fukai, R.W. Alexander, M. Akers, K.K. Griendling, p38 Mitogen-activated protein kinase is a critical component of the redox-sensitive signaling pathways activated by angiotensin II. Role in vascular smooth muscle cell hypertrophy, J. Biol. Chem. 273 (1998) 15022–15029.
[41] P.P. Roux, J. Blenis, ERK and p38 MAPK-activated protein kinases: A family of protein kinases with diverse biological functions, Microbiol. Mol. Biol. Rev. 68 (2004) 320–344.
[42] G. Bahrenberg, I. Behrmann, A. Barthel, P. Hekerman, P.C. Heinrich, H.G. Joost, W. Becker, Identification of the critical sequence elements in the cytoplasmic domain of leptin receptor isoforms required for Janus kinase/signal transducer and activator of transcription activation by receptor heterodimers, Mol. Endocrinol. 16 (2002) 859–872.
[43] A.M. Wallace, A.C. McMahon, C.J. Packard, A. Kelly, J. Shepherd, A. Gaw, N. Sattar, Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS), Circulation 104 (2001) 3052–3056.
[44] A. Singhal, I.S. Farooqi, T.J. Cole, S. O'Rahilly, M. Fewtrell, M. Kattenhorn, A. Lucas, J. Deanfield, Influence of leptin on arterial distensibility: A novel link between obesity and cardiovascular disease? Circulation 106 (2002) 1919–1924.
[45] J. Sundell, R. Huupponen, O.T. Raitakari, P. Nuutila, J. Knuuti, High serum leptin is associated with attenuated coronary vasoreactivity, Obes. Res. 11 (2003) 776–782.
[46] R. Wolk, P. Berger, R.J. Lennon, E.S. Brilakis, B.D. Johnson, V.K. Somers, Plasma leptin and prognosis in patients with established coronary atherosclerosis, J. Am. Coll. Cardiol. 44 (2004) 1819–1824.
[47] P. Piatti, C. Di Mario, L.C. Monti, G. Fragasso, F. Sgura, A. Caumo, E. Setola, P. Lucotti, E. Galluccio, C. Ronchi, A. Origgi, I. Zavaroni, A. Margonato, A. Colombo, Association of insulin resistance, hyperleptinemia, and impaired nitric oxide release with in-stent restenosis in patients undergoing coronary stenting, Circulation 108 (2003) 2074–2081.