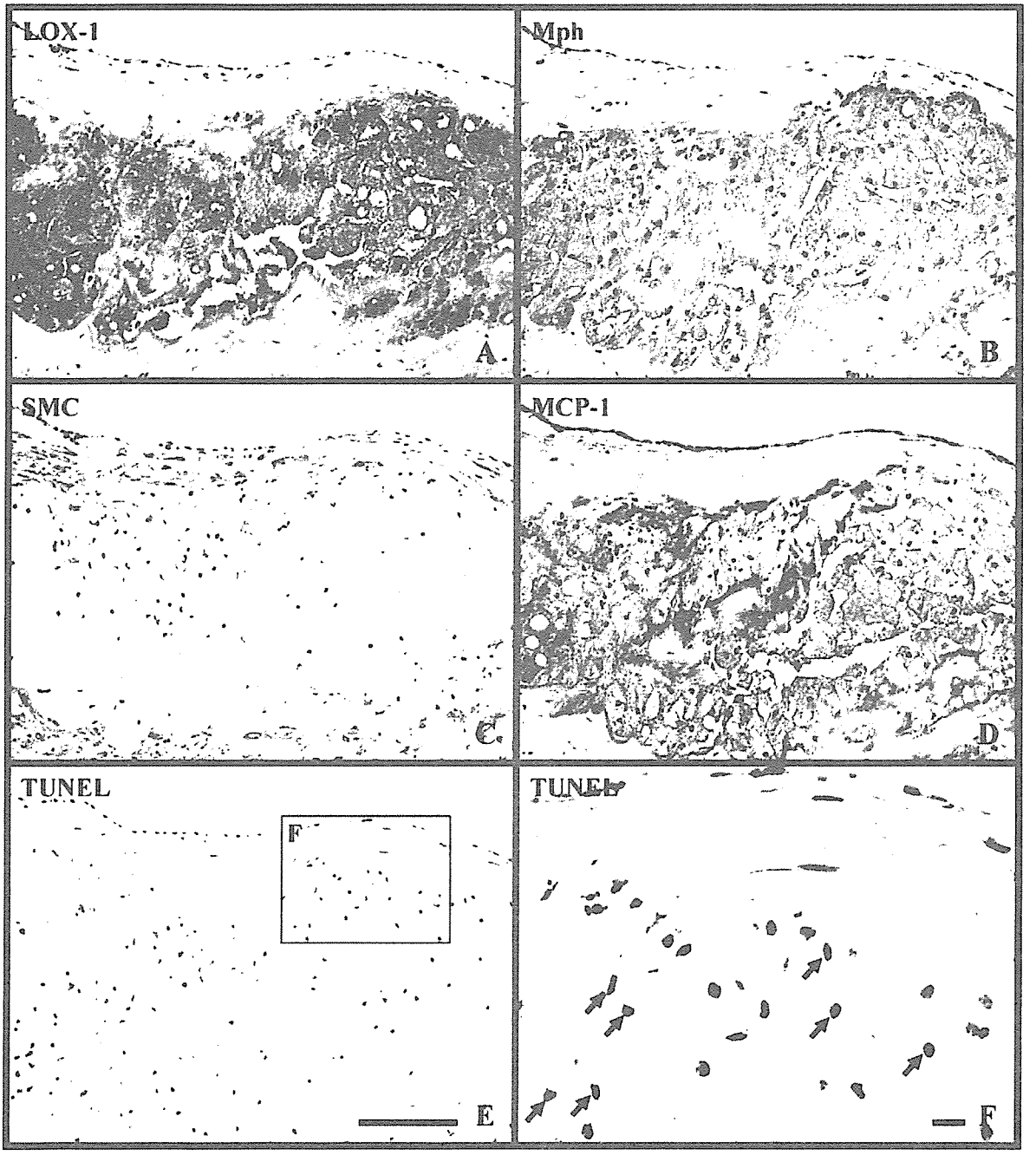

Fig. 5. Typical microscopic images of immunohistochemical staining for LOX-1 (A), macrophages (B), smooth muscle cells (C), and MCP-1 (D), and TUNEL staining (E) in the lipid core area of unstable plaque. Panel (F) showed a higher magnification image of the area indicated as a square in panel (E). Arrows indicate TUNEL-positive nuclei. Bar = 100 μm, magnifications × 150 (A–E), × 450 (F).

LOX-1 was reported to be expressed in endothelial cells and macrophages in early atherosclerotic lesions of aortas from 2-month-old WHHL rabbits [18]. In the present study, however, we used the aortas of mature rabbits to examine advanced atherosclerotic plaques. LOX-1 in these advanced plaques was colocalized with smooth muscle cells in fibromuscular caps, macrophages, endothelial cells and Azan-positive areas. Smooth muscle cells and macrophages in advanced atherosclerotic plaques in humans similarly express LOX-1 [19], thus indicating the histological similarity of atherosclerotic plaques between humans and this animal model. In this study, LOX-1 expression was also observed in Azan-positive areas. This might result from soluble LOX-1 bound to extracellular matrix proteins [26]. In

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destabilized plaques, smooth muscle cells might disappear by apoptosis after expressing abundant LOX-1 and releasing large amounts of soluble LOX-1.

We also compared LOX-1 expression with other two crucial factors in the destabilization of atherosclerotic plaques, such as MCP-1 expression and apoptotic events. MCP-1 appears to be a key molecule regulating atherosclerotic plaque instability by recruiting monocytes/macrophages into the plaques and thus eliciting proinflammatory responses. It has been reported that LOX-1-mediated uptake of Ox-LDL induces MCP-1 expression in cultured cells [7]; however, it remained unknown whether LOX-1 expression is associated with MCP-1 expression in vivo. In the present study, we provide evidence, for the first time, that LOX-1 expression is co-localized with MCP-1 in the lipid core area. Furthermore, TUNEL-positive apoptotic cells were also co-localized with LOX-1 expression in the lipid core area. Apoptosis of foam cells and macrophages is thought to contribute to the formation of the acellular lipid core [27]. Taken together, LOX-1 is suggested to be involved in atherosclerotic plaques rupture by regulating MCP-1 expression and apoptotic events, in addition to MMP expression.

Animal models suitable for studying the spontaneous rupture of unstable plaques have yet to be established. In this hypercholesterolemic rabbit model, the rupture of atherosclerotic plaques was reported to be undetectable in aortic lesions and most coronary lesions [28]. However, rupture-prone unstable plaques characterized in humans as those consisting of thin fibromuscular caps and large lipid cores with numerous macrophages were similarly observed in both aortic (Fig. 2) and coronary lesions in this animal model [28]. Atherosclerotic plaques with these histological characteristics appear to be causative of acute coronary syndrome in humans [4,29,30]. In the present study, therefore, we utilized atherosclerotic plaques consisting of fibromuscular caps and lipid cores in this rabbit aortas as a model to analyze plaque instability, focusing on the components of plaques and expression of LOX-1 and MMP-9. The relationship between LOX-1 expression and histological instability may also be observed in human coronary plaques, although this point should be clarified in the future. In fact, Ox-LDL, the ligand of LOX-1, has been shown to be more abundantly accumulated in coronary atherosclerotic plaques of patients with acute coronary syndrome than stable angina pectoris [31].

Aikawa et al. demonstrated the stabilization of atherosclerotic lesions resulting from lipid lowering on cessation of cholesterol-feeding and statin administration, using the aortas of rabbits with diet-induced hypercholesterolemia and ballon-injury [32], as well as genetically hypercholesterolemic WHHL rabbits [33]. In the present study, we did not include any interventions, such as lipid lowering or balloon injury; however, atherosclerotic plaques with a variety of histological characteristics were observed in the same individual WHHLMI rabbits. Therefore, we compared these histological characteristics with the expression of LOX-1 and MMP-9, both of which appear to be key molecules regulating plaque instability.

In addition, lipid lowering may also stabilize atherosclerotic plaques and may reduce LOX-1 expression in this model, as well as in humans, because lipid lowering can reduce the accumulation of Ox-LDL in arterial walls which induces LOX-1 expression [11]. Furthermore, statins directly suppress LOX-1 expression in cultured vascular cells [34]; therefore, statins can also reduce LOX-1 expression in vivo independently of Ox-LDL levels. These points, which could provide clinically important and intriguing insights, should be clarified in the future.

In summary, the present study demonstrated that LOX-1 expression is associated with the instability of atherosclerotic plaques in a hypercholesterolemic animal model. Thus, LOX-1 may be a therapeutic target to prevent the rupture of atherosclerotic plaques, since the serum level of soluble LOX-1 has recently been shown to have diagnostic value for acute coronary syndrome [35].

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Leptin induces elongation of cardiac myocyte and causes eccentric left ventricular dilatation with compensation

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Short Title: Leptin induces elongation of cardiomyocytes

Abstract

One of the major manifestations of obesity is an increased production of the adipocyte-derived 16-kDa peptide leptin, which acts mainly on hypothalamic leptin receptors. Leptin receptors are widely distributed in various tissues, including the heart. While increased plasma leptin levels have been reported in patients with congestive heart failure, systemic alterations induced by obesity can affect cardiac hypertrophy and the direct effects of leptin on cardiac structure and function still remain to be determined. We first exposed primary cardiac myocytes from neonatal rats to leptin for 48 hours. This resulted in a significant increase in myocyte long-axis length ($p<0.05$ at 50ng/mL), but not in the short-axis width. Leptin induced the rapid phosphorylation of STAT3 and its DNA binding in cardiac myocytes. Administration of a JAK2 inhibitor, AG490, completely inhibited all of these effects by leptin. Furthermore, we examined the effect of continuous infusion of leptin for four weeks following myocardial infarction (MI) in mice. Echocardiography demonstrated that left ventricular fractional shortening in the leptin-infused group (28.4±2.8%) was significantly higher than that in the PBS-infused group (18.4±2.2%) following MI. Interestingly, LV diastolic dimension in the leptin-infused group (4.56±0.12 mm) was also higher than that in the PBS-infused group (4.13±0.09 mm). These results demonstrate that leptin induces the elongation of cardiac myocytes via a JAK/STAT pathway and chronic leptin infusion causes eccentric dilatation with augmented systolic function after myocardial infarction.

Key Words: leptin, obesity, hypertrophy, heart failure, signal transduction

Introduction

A growing epidemic of overweight and obesity is affecting many countries worldwide. Obesity, even when uncomplicated by hypertension or diabetes, is frequently associated with left ventricular (LV) hypertrophy as assessed through ECG criteria or echocardiography [6,7,11,12]. Previous reports have strongly suggested that LV hypertrophy associated with obesity is eccentric in nature [3,5,30]. In moderate to severe cases of obesity, this may lead to LV diastolic dysfunction. LV systolic dysfunction may also occur if wall stress remains high because of inadequate hypertrophy [2]. Therefore, identifying the key mechanism for the development of cardiac hypertrophy in obese patients may offer unique insights into the understanding of obesity-induced changes in cardiovascular function.

One of the major manifestations of obesity is an increased production of the adipocyte-derived 16-kDa peptide leptin. Leptin regulates cellular homeostasis and glycemic control [31]. While leptin was initially described as an adipocyte-derived protein with expression and secretion restricted to adipose tissue, recent reports have demonstrated that it is also locally expressed in other tissues [8, 15]. Leptin receptor isoforms have been shown to be expressed in the myocardium and in isolated cardiac myocytes [19]. This suggests that leptin has specific effects on the heart. Several recent studies have supplied evidence that leptin directly induces hypertrophy in cardiomyocytes in culture [13, 20, 26, 29]. The observation that elevated plasma leptin levels are found in patients with LV hypertrophy or congestive heart failure suggests that leptin may play a role in human cardiac hypertrophy [17, 18, 21]. On the other hand, one report suggested that leptin mediates 'antihypertrophic' effects [4]. Therefore, the direct effects of leptin on myocardial cell morphology and cardiac function still remain to be determined.

In this report we provide evidence for a potential link between leptin and LV eccentric hypertrophy by demonstrating that leptin induces cardiac myocyte elongation *in vitro* and eccentric dilatation with augmented systolic function after myocardial infarction (MI) *in vivo*.

Materials and Methods

Immunocytochemistry and measurement of cell size The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Primary neonatal rat ventricular cardiac myocytes were prepared from SD rats as previously described [9]. The cardiac myocytes were grown on flask-style chambers with glass slides (Nalgen Nunc, Naperville, IL) and then stimulated with saline or 5-500 ng/ml of leptin in serum-free medium for 48 h. The cells were then fixed with 3% formaldehyde in phosphate-buffered saline for 15 min at room temperature. Immunocytochemical staining for β-MHC (myosin heavy chain) was performed using the indirect immunoperoxidase method. The anti-cardiac β-MHC polyclonal antibody (NovoCastra Laboratories Ltd., Newcastle, UK) was used at a dilution of 1:50. A total of 50 myocardial fibers were selected randomly from cardiac myocytes stained with anti-β-MHC antibody, and the surface areas of these cells were measured semi-automatically with the aid of an image analyzer (LUZEX 3U; Nikon, Tokyo, Japan) as previously described [10]. Cell long-axis length was defined as the maximum longitudinal extension of individual cells. Maximum short-axis width was measured perpendicular to the axis that defined cell length. Immunocytochemical staining for STAT3 was performed using the indirect immunofluorescence method. The cells were fixed with Bouin's solution for 10 min at room temperature and subsequently autoclaved for 10 min at 121 °C in 10 mM citrate, pH 6.0. The cells were incubated with anti-STAT3 monoclonal antibody (Santa Cruz Biotechnology) at a dilution of 1:100. STAT3 signals were detected with anti-mouse FITC-conjugated secondary antibody at a dilution of 1: 75 for 45 min.

Analysis of the phosphorylation state of STAT3, ERK1/2, p38 and JNKs Cells were lysed with 2x Laemmli loading buffer (2% SDS, 20% glycerol, 0.04 mg/ml bromophenol blue, 0.12 M Tris-HCl, pH 6.8, and 0.28 M β-mercaptoethanol) (150 μl/35-mm dish). Filters were probed with anti-phosphospecific STAT3, ERK1/2, p38 and JNK antibody (New England Biolabs, Beverly, MA). To normalize for protein loading after immunoprecipitation, blots were stripped by incubation in 62.5 mM Tris-HCl, pH 6.8, 100 mM β-mercaptoethanol, and 2% SDS for 30 min at 50 °C, washed twice with

phosphate-buffered saline and 0.05% Tween, and then probed with an antibody that recognizes both phosphorylated and non-phosphorylated forms of STAT3, ERK1/2, p38 and JNK (New England Biolabs, Beverly, MA). The mean of the integrated density obtained from three independent files was used as a representative value for each band.

RT-PCR RT-PCR was used to analyse the expression of rat isoform-specific Ob-Ra and Ob-Rb as described previously [23]. For real-time PCR, the reaction was performed with a SYBR® Green PCR master mix (Applied Biosystems), and the products were analyzed with a thermal cycler (ABI Prism®7900HT sequence detection system). Levels of GAPDH transcript were used to normalize cDNA levels. Gene-specific primers were as follows;

Rat Ob-Ra sense : 5' TGGCCCATGAGTAAAGTGAATGCTG3',
Rat Ob-Ra antisense: 5'TCAAAGAGTGTCCGCTCTCTTTTGG3',
Rat Ob-Rb sense: 5' TGGCCCATGAGTAAAGTGAATGCTG3',
Rat Ob-Rb antisense: 5' TCTTCTGAAACTGGTTCAGGCTCCA3'.
ANF sense; 5'CGTGCCCCGACCCACGCCAGCATGGGCTCC3'
ANF antisense; 5'GGCTCCGAGGGCCAGCGAGCAGAGCCCTCA3'
Mouse Ob-Ra and Ob-Rb sense; 5'TGTTTTGGGACGATGTTCCA3'
Mouse Ob-Ra antisense: 5'ATTGGGTTCATCTGTAGTGGTCATG3',
Mouse Ob-Rb antisense: 5' GCTTGGTAAAAAGATGCTCAATG3'.
GAPDH sense; 5'TTGCCATCAACGACCCCTTC3'
GAPDH antisense; 5'TTGTCATGGATGACCTTGGC3'

Electrophoretic mobility shift assays (EMSAs) We used double-stranded oligonucleotides (purchased from Santa Cruz Biotechnology, Inc.) that contained STAT3 binding sites and mutant STAT3 binding motif for the assay. We also used oligonucleotides with an SP1 binding site as a control probe. EMSAs were carried out at 4°C for 20 min in 15-μl reaction mixtures containing 10 μg of nuclear extract, 0.25 ng (>20,000 cpm) of radiolabeled double-stranded oligonucleotide, 500 ng of poly(dI-dC), 5 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.5 mM dithiothreitol, 37.5 mM KCl, and 4% Ficoll 400. For cold competition experiments, a 100-fold excess of unlabeled competitor oligonucleotide was included in the binding reaction mixture. Protein-DNA complexes

were separated by electrophoresis on 4% nondenaturing polyacrylamide gels in 0.25x TBE (1x TBE is 100 mM Tris, 100 mM boric acid, and 2 mM EDTA) at 4°C.

Experimental MI, implantation of micro-osmotic pumps and transthoracic echocardiography C57/BL6 mice were anesthetized with 1.0 to 1.5% isoflurane and open-chest coronary artery ligation was performed. MI was induced by ligating the left anterior descending coronary artery. During this procedure, micro-osmotic pumps (DURECT Co.) were implanted subcutaneously in the intrascapular region of each mouse. The pumps infused solutions at a rate of 2.5 μl/h for 28 days. The reservoir of each pump was preloaded with 2000 μl of either sterile PBS or recombinant mouse leptin (R&D Systems) (1.54 μg/μl), to give a leptin infusion rate of approximately 0.32 μg/g/day. The dose of leptin was chosen to not increase blood pressure. The cardiac functions of mice were evaluated non-invasively by echocardiography after 4 weeks. The animals were anesthetized with ketamine (50 μg/g of body weight) and xylazine (2.5 mg/g). Transthoracic echocardiography was performed with a cardiac ultrasound recorder (Toshiba Power Vision, Tokyo, Japan) using a 7.5-MHz transducer. After the acquisition of high-quality two-dimensional images, M mode images of the left ventricle were recorded. LV end diastolic (LVDD) and end systolic (LVESD) internal dimensions were measured according to the leading edge-to-leading edge convention adopted by the American Society of Echocardiography. Percent fractional shortening (%FS) was calculated as follows: %FS = [(LVDD - LVESD)/LVDD] x 100. At least three independent M mode measurements were obtained per animal by an examiner who was blinded to the genotype of the animal. Plasma leptin concentration was measured by a mouse leptin immunoassay kit (R&D Systems).

Statistical analysis Data are presented as means ± S.E. Statistical comparisons were performed using unpaired two-tailed Student's *t* tests or an analysis of variance with Scheffe's test where appropriate, with a probability value of less than 0.05 taken to indicate significance.

Results

Effect of leptin on primary cardiomyocytes We exposed primary cardiac myocytes from neonatal rats to leptin at concentrations of 0, 5, 50, and 500ng/ml for 48 hours. As shown in Fig. 1A, brown signals, which indicated the presence of β-MHC, were observed in both saline- and leptin-stimulated cardiac myocytes. Cardiac myocytes that had been stimulated with leptin at a concentration of 50ng/ml, which is usually observed in obese patients [14], showed increases in cell size and myofibrillar organization compared with saline-stimulated cells. As shown in Fig. 1B, leptin stimulation (50ng/ml) resulted in a significant increase in the myocyte surface area (*left panel*) and long-axis length (*middle panel*), but not in the short-axis length (*right panel*). An increase in the leptin concentration from 50ng/ml to 500ng/ml resulted in a slight but not significant increase in the myocyte cell surface area. Thus, a physiological concentration of leptin (50ng/ml) was sufficient to induce the elongation of cardiac myocytes in culture. We also measured the mRNA levels of atrial natriuretic factor (ANF), which is closely associated with cardiomyocyte hypertrophy, and GAPDH after leptin treatment. As shown in Fig. 1C, leptin stimulation (50ng/ml and 500ng/mL) resulted in a significant increase in the relative expression level of ANF/GAPDH.

Leptin induces STAT3 activation in cardiac myocytes To investigate the intracellular pathway for leptin signaling in cardiac myocytes, we first examined the expression of two leptin receptor isoforms, short (Ob-Ra) and long (Ob-Rb) forms, by RT-PCR. A previous study in neonatal rat ventricular myocytes demonstrated the predominant expression of Ob-Ra and leptin-induced activation of ERK1/2 and p38 at 5 minutes after stimulation [20]. In contrast to that study, we observed mRNA expression of Ob-Rb, which is linked to JAK/STAT signaling, in addition to that of Ob-Ra under our experimental conditions (Fig. 2A). Next, we examined the phosphorylation (activation) of MAP kinases (ERK1/2, p38, and JNKs) and STAT3 in cardiac myocytes by Western blotting. We did not recognize the significant activation of ERK1/2, p38, or JNKs at 10 minutes after treatment with 50ng/ml of leptin (Fig. 2B). As shown in Fig. 2C, the phosphorylated form of STAT3 was increased in leptin-stimulated cardiac myocytes (*lanes 2-4*) compared to saline-stimulated cells (*lane 1*), compatible with the expression

of Ob-Rb. Activation was evident as early as 5 minutes after stimulation (*lane 2*). These findings suggest that leptin stimulation predominantly activated the JAK/STAT pathway in cardiac myocytes.

Leptin induces nuclear translocation of STAT3 in cardiac myocytes To further examine the activation of STAT3 by leptin in cardiac myocytes, we performed an immunofluorescence microscopic analysis. As shown in Fig. 3, STAT3, which is indicated by the green signal of FITC, was detected in the cytoplasm of nearly all saline-stimulated cardiac myocytes (*upper left panel*). However, stimulation of cardiac myocytes with leptin for 15 minutes markedly changed this localization and caused the nuclear translocation of STAT3 (*upper right panel*). By double staining with an antibody against cardiac β-MHC, we confirmed that these cells are myocytes (*middle panels*). These findings provide further evidence for the leptin-induced activation of the JAK-STAT pathway in cardiac myocytes.

Leptin increases the DNA-binding activity of STAT3 To determine whether leptin modulates the DNA-binding activity of STAT3 in cardiac myocytes, EMSAs were performed. Nuclear extracts were prepared from neonatal cardiac myocytes that had been stimulated with leptin in the absence or presence of AG490 (1 μM), a specific inhibitor of JAK2, or that had been treated with saline as a control. These extracts were probed with a radiolabeled double-stranded oligonucleotide containing the consensus STAT3 site. As shown in Fig. 4A, competition EMSAs revealed that a retarded band represented specific binding, as evidenced by the fact that it was competed out by an excess of unlabeled STAT3 oligonucleotide (*lane 4*), but not by the same amount of an oligonucleotide containing the STAT3 site with a mutation (*lane 5*). The amount of the specific complex containing STAT3 was markedly increased in nuclear extracts from leptin-stimulated myocytes (*lane 2*) compared to those from saline-treated cells (*lane 1*). Notably, the leptin-stimulated increase in STAT3-binding activity was almost completely blocked by 1 μM AG490 (Fig. 4A, *lane 3* and Fig. 4C). In contrast, SP1-binding activities were not altered by either leptin or leptin plus AG490 (Fig. 4B).

AG490 inhibits cardiomyocyte hypertrophy induced by leptin We tested whether AG490 affects leptin-induced myocardial cell hypertrophy. Neonatal rat ventricular myocytes were preincubated with or without 1μM AG490 for 1 h. Activation of STAT3 was evaluated by Western blot analysis as described above. As shown in Fig. 5A, AG490 inhibited leptin-induced activation of STAT3 (compare *lanes 1, 3* and *4*), but did not affect the phosphorylation status in the basal state (compare *lanes 1* and *2*). As shown in Fig. 5B, the same dose of AG490 completely inhibited the increase in the myocardial cell surface area induced by leptin. AG490 alone did not affect the area in saline-stimulated cardiac myocytes. These data demonstrate that AG490 can selectively suppress the hypertrophic response induced by leptin.

Effect of chronic leptin infusion on cardiac structure and function following MI To determine the effect of chronic leptin infusion on cardiac structure and function following MI, 12-week-old mice were subjected to MI or sham operation. Each mouse was then subcutaneously fitted with an Alzet mini-osmotic pump that delivered either PBS or 400 ng/hr (0.32 μg/g/day) of leptin for 4 weeks. The plasma leptin concentration significantly reduced after myocardial infarction in mice infused with PBS. Chronic infusion of leptin significantly increased the plasma leptin level in both sham-operated and MI mice (Fig. 6A). At this concentration, leptin infusion did not affect food intake or body weight during examination. The heart weight (HW)/ body weight (BW) ratio and the relative expression level of ANF were significantly enhanced after myocardial infarction. The relative expression level of ANF/GAPDH in infarcted hearts in leptin-infused mice was significantly higher than that in PBS-infused mice following MI (Fig. 6C). Blood pressure and heart rate were similar between the PBS- and leptin-infused groups (Fig 7A). As shown in Fig 7B, echocardiography demonstrated that there was no difference in LV dimensions or systolic functions between leptin- and PBS-infused mice with sham-operation (white bars). In MI-mice (black bars), however, both LV chamber diameter in end-diastole (LVEDD, *left panel*) and fractional shortening (FS, *right panel*) were significantly higher in the leptin-infused group compared with the PBS-infused group. Thus, chronic leptin infusion resulted in eccentric dilatation with augmented systolic function following MI. To identify a possible link between leptin infusion and

LV dilatation, we measured the expression levels of Ob-Ra and Ob-Rb in these mouse hearts. Interestingly, while Ob-Rb expression was significantly enhanced after myocardial infarction (Fig. 7C), there was no change in the level of Ob-Ra.

Discussion

Leptin is an adipocyte-derived peptide, the production of which is increased in patients with obesity. In addition to its action as a neuropeptide, it has become clear that leptin plays many roles as a growth factor in several cell types including cardiac myocytes [19, 20, 26, 29]. Clinical evidence has implicated an increased plasma leptin level as a potential independent risk factor for coronary heart disease, and leptin has been associated with cardiac hypertrophy and heart failure [17, 18, 21, 27]. Therefore, it has become increasingly important to understand the effect of obesity-associated hyperleptinemia on cardiovascular systems.

Leptin exerts its effects through short (Ob-Ra) and long (Ob-Rb) receptor isoforms. It has been suggested that these two isoforms are coupled with distinct downstream signaling cascades; Ob-Ra is associated with MAP kinases, and Ob-Rb is coupled with JAK/STAT. A previous study demonstrated the predominant expression of Ob-Ra in neonatal rat cardiomyocytes and the leptin-induced activation of MAP kinases [20]. Another study in human ventricular myocytes showed the leptin-induced activation of both MAP kinases and JAK/STAT as well as the expression of both Ob-Ra and Ob-Rb in these cells. The present study demonstrated that leptin induced activation of the JAK/STAT pathway in neonatal rat cardiac myocytes and that this pathway is required for elongation of these cells. Although we detected both Ob-Ra and Ob-Rb by RT-PCR, we did not detect the significant activation of MAP kinases after exposure to leptin for 10 minutes. Differences in the technique for preparing primary cardiac myocytes and their culture conditions might explain the discrepancy in the in vitro experimental results regarding receptor subtype expression and signaling cascades.

It has been shown that the gp130/ leukemia inhibitory factor (LIF) receptor β-dependent cytokines, cardiotrophin-1 and LIF, induce an increase in myocyte size characterized by a marked increase in cell length, but little or no change in cell width [24, 28]. Compatible with the fact that Ob-Rb is considerably similar to gp130 and LIF receptor, leptin had a more pronounced effect on cardiomyocyte length than on cardiomyocyte width. Myocytes from heart with eccentric hypertrophy, which is often induced by volume overload, exhibit the assembly of sarcomeric units in series, while those from heart with pressure overload-induced concentric hypertrophy show the

parallel assembly of sarcomeric units [1, 16, 25]. Our results in vitro also suggest that obesity-induced hyperleptinemia is, at least in part, attributable to eccentric hypertrophy. This finding prompted us to study the in vivo effect of leptin on cardiac morphology and function.

Previously, it was reported that infusion with leptin at 1 μg/kg/min caused sustained increases in blood pressure [22]. Since hypertension can induce cardiac hypertrophy by itself, we examined the effects of leptin on cardiac function at a lower concentration (400ng/ hour = 0.32 μg/kg/min) than that used in the previous report. Chronic infusion of leptin at this concentration did not cause any changes in food intake, blood pressure or heart rate, suggesting that activation of the sympathetic nervous system did not occur under our conditions. We demonstrated here that, in MI-mice, both LV diastolic dimension and fractional shortening in the leptin-infused group were significantly higher than those in the PBS-infused group. Therefore, leptin induces the enhancement of LV systolic function as well as LV eccentric dilatation after MI without increase in HW/BW ratio in vivo. In this experiment, we also demonstrated that the expression level of Ob-Rb is augmented in hypertrophied heart after MI. Therefore, it is possible that the JAK/STAT pathway may be especially enhanced through Ob-Rb after MI.

A previous study suggested that leptin mediates 'antihypertrophic' effects [4]. In this study, infusion of leptin into ob/ob mice, which lack leptin and exhibit left ventricular hypertrophy, reversed the increase in left ventricular wall thickness. However, there is a possibility that the original hypertrophy in these mice may be the result of systemic alterations caused by a chronic lack of leptin. Moreover, the ability of leptin to reverse hypertrophy most likely reflects the correction of whole-body physiological parameters that influence cardiac structure rather than a direct effect on myocytes. Therefore, based on a consideration of our data together with those in previous works [13, 20, 26, 29], we believe that the acute direct effects of leptin on cardiac myocytes may be pro-hypertrophic.

Although the present study suggests that leptin may contribute to the development of eccentric LV dilation in obese patients, it is unlikely that a single cytokine or growth factor can explain all of the changes in cardiac morphology and function in these patients. However, clarifying the precise roles of leptin in the regulation of cardiac function may

aid in the development of new therapeutic strategies for heart failure, especially in obese patients.

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