Leptin Attenuates Cardiac Contraction in Rat Ventricular Myocytes

Role of NO

Marvie W. Nickola,* Loren E. Wold,* Peter B. Colligan, Guei-Jane Wang, Willis K. Samson, Jun Ren

Abstract—Obesity is commonly associated with impaired myocardial contractile function. However, a direct link between these 2 states has not yet been established. There has been an indication that leptin, the product of the human obesity gene, may play a role in obesity-related metabolic and cardiovascular dysfunctions. The purpose of this study was to determine whether leptin exerts any direct cardiac contractile action that may contribute to altered myocardial function. Ventricular myocytes were isolated from adult male Sprague-Dawley rats. Contractile responses were evaluated by use of video-based edge detection. Contractile properties analyzed in cells electrically stimulated at 0.5 Hz included peak shortening, time to 90% peak shortening, time to 90% relengthening, and fluorescence intensity change. Leptin exhibited a dose-dependent inhibition in myocyte shortening and intracellular Ca\(^{2+}\) change, with maximal inhibitions of 22.4% and 26.2%, respectively. Pretreatment with the NO synthase inhibitor L-NAME (100 \(\mu\)mol/L) blocked leptin-induced inhibition of both peak shortening and fluorescence intensity change. Leptin also stimulated NO synthase activity in a time- and concentration-dependent manner, as reflected in the dose-related increase in NO accumulation in these cells. Addition of an NO donor (S-nitroso-N-acetyl-penicillamine [SNAP]) to the medium mimicked the effects of leptin administration. In summary, this study demonstrated a direct action of leptin on cardiomyocyte contraction, possibly through an increased NO production. These data suggest that leptin may play a role in obesity-related cardiac contractile dysfunction. (Hypertension. 2000;36:501-505.)

Key Words: hormones ■ myocytes ■ calcium ■ nitric oxide

Obesity is associated with an increased incidence of cardiovascular diseases, such as hypertension, stroke, and congestive heart failure. 1 Although it appears that cardiac function may be normal or enhanced in the early stage of obesity, 2 cardiac hypertrophy and compromised ventricular function develop as a result of the increased cardiac pressure and/or volume overload in obesity. The reduced ventricular function thus leads to impairment of ejection fraction/rate, fractional shortening, and diastolic compliance. 3,4 The mechanism(s) responsible for the obesity-induced cardiac alteration remains uncertain but may be related to salt sensitivity, insulin resistance, and sympathetic activation. 5

Leptin, the product of the obesity gene (ob), 6 is a peptide hormone expressed in adipose tissue. Leptin regulates body weight through the inhibition of food intake and promotion of energy expenditure. 7,8 The leptin receptor has several alternatively spliced variants. One of which, the Ob-Rb variant, is believed to be functional and has been shown to exist in various tissues, including the heart. 9 Recent studies have shown that leptin increases in insulin-resistant states, such as obesity and hypertension. 10–12 Obesity and hyperinsulinemia are considered the major stimulators of leptin production. 13,14 Nevertheless, no direct relationship between plasma leptin levels and cardiovascular function has been established. Leptin has been shown to increase heart rate and blood pressure through the stimulation of sympathetic nervous system activity. 15,16 Furthermore, fasting plasma leptin levels are associated with increased myocardial wall thickness, independent of body composition and blood pressure levels. 17 However, no evidence for a direct effect of leptin on cardiac contractile function has been reported. To address this possibility, we evaluated the effect of leptin on cell shortening, intracellular Ca\(^{2+}\), and NO synthase (NOS) activity in myocytes isolated from adult rat ventricles.

Methods

Isolation of Ventricular Myocytes

The experimental procedures were approved by the animal investigation committee of the University of North Dakota. Single ventri-
ular myocytes were isolated from adult male Sprague-Dawley rats (200 to 225 g) as described previously. Briefly, the animals were euthanized, and their hearts were rapidly removed and perfused (at 37°C) with oxygenated (5% CO2/95% O2) Krebs-Henseleit bicarbonate (KHB) buffer (mmol: NaCl 118, KCl 4.7, CaCl2 1.25, MgSO4 1.2, KH2PO4 1.2, NaHCO3, 25, HEPES 10, and glucose 11.1, pH 7.4). Hearts were subsequently perfused with a nominally Ca2+-free KHB buffer for 2 to 3 minutes until spontaneous contractions ceased, followed by a 20-minute perfusion with Ca2+-free KHB containing 223 U/mL collagenase (Worthington Biochemical Corp) and 0.1 mg/mL hyaluronidase (Sigma Chemical Co). After perfusion, the left ventricle was removed, minced, and incubated with the fresh enzyme solution (Ca2+-free KHB containing 223 U/mL collagenase) for 3 to 5 minutes. The cells were further digested with 0.02 mg/mL trypsin (TR90), and maximal velocities of shortening (PS), time to 90% PS (TPS), time to 90% relengthening (TR), and maximal velocities of relengthening (+dL/dt) were determined after 15 minutes of incubation of the myocytes with leptin (0.1 to 1000 nmol/L).

Mechanical properties of ventricular myocytes were assessed by using a video-based edge-detection system (IonOptix) as described. In brief, coverslips with cells attached were placed in a chamber mounted on the stage of an inverted microscope (Olympus X-70) and superfused (200 to 225 g) as described previously. Briefly, the animals were euthanized, and their hearts were rapidly removed and perfused (at 37°C) with oxygenated (5% CO2/95% O2) Krebs-Henseleit bicarbonate (KHB) buffer (mmol: NaCl 118, KCl 4.7, CaCl2 1.25, MgSO4 1.2, KH2PO4 1.2, NaHCO3, 25, HEPES 10, and glucose 11.1, pH 7.4). Hearts were subsequently perfused with a nominally Ca2+-free KHB buffer for 2 to 3 minutes until spontaneous contractions ceased, followed by a 20-minute perfusion with Ca2+-free KHB containing 223 U/mL collagenase (Worthington Biochemical Corp) and 0.1 mg/mL hyaluronidase (Sigma Chemical Co). After perfusion, the left ventricle was removed, minced, and incubated with the fresh enzyme solution (Ca2+-free KHB containing 223 U/mL collagenase) for 3 to 5 minutes. The cells were further digested with 0.02 mg/mL trypsin (TR90), and maximal velocities of shortening (PS), time to 90% PS (TPS), time to 90% relengthening (TR), and maximal velocities of relengthening (+dL/dt) were determined after 15 minutes of incubation of the myocytes with leptin (0.1 to 1000 nmol/L).

Effect of Leptin on Myocyte Shortening (PS)

Acute exposure (up to 15 minutes) of leptin did not affect resting myocyte cell length over the range of concentrations tested. A representative trace depicting the effect of leptin (100 nmol/L) on myocyte shortening is shown in Figure 1A. At the end of a 15-minute exposure to this concentration of leptin, myocyte shortening was decreased by ~15%. Leptin exhibited little effect on the duration of shortening (TPS) and relengthening (TR). Leptin (0.1 to 1000 nmol/L) elicited a concentration-dependent depression of myocyte shortening, with a maximal inhibition of 22.4% obtained at 1000 nmol/L. The concentration at which leptin displayed 50% maximal response (EC50) was 10.1 nmol/L. The depressive effect of leptin on cell shortening was maximal within 8 minutes of exposure and was partially reversible on washout (data not shown). The inhibitory effect of leptin was not associated with any impact on the duration of either TPS or TR. The +dL/dt values were not affected, with the exception of +dL/dt at the highest dose of leptin (Table).

Effect of Leptin on Intracellular Ca2+ Transients

To determine whether leptin-induced inhibition of myocyte shortening was due to the reduced availability of intracellular free Ca2+, [Ca2+], in response to electrical stimuli in the presence of various concentrations of leptin was examined. Representative traces of intracellular Ca2+ transients shown in...
Leptin has been shown to increase serum levels of NO, an important regulator in the cardiovascular system. Constitutive and inducible NOS are both present in cardiac myocytes. To examine the potential mechanism of action for leptin, the effect of leptin on PS and intracellular Ca\(^{2+}\) transients was reexamined in the presence of the NOS inhibitor L-NAME (100 \(\mu\)mol/L). L-NAME alone had no effect on PS and intracellular Ca\(^{2+}\) transients over 30 minutes (data not shown). As shown in Figures 1B and 2B, the leptin-induced decrease in both PS and intracellular Ca\(^{2+}\) transients was completely abolished by L-NAME. Leptin (10 nmol/L) in the presence of L-NAME even elicited a small but significant positive effect on DFFI. These data suggest that leptin may exert its inhibition on PS and intracellular Ca\(^{2+}\) transients, at least in part, through NO production.

**Effect of Leptin on NOS Activity**

To further ensure the potential involvement of NO in leptin-induced cardiac contractile action, the effect of leptin on NOS activity was measured directly. Data presented in Figure 3 indicate that leptin elicited a concentration- and time-dependent increase in NOS activity in ventricular myocytes (Figure 3). Furthermore, incubation of myocytes with the NO donor S-nitroso-N-acetyl-penicillamine (SNAP, 10 to 100 \(\mu\)mol/L) for 15 minutes elicited a depression of myocyte shortening, indicating that elevated NO production is associated with the depression of cardiac contraction, as reported previously (Figure 4).

**Discussion**

The present study demonstrated that the \(ob\) gene product leptin depresses ventricular myocyte shortening and intracel-
Compromised cardiac systolic function has been reported in Zucker obese rat hearts and, recently, at the isolated ventricular myocyte level in the same animal model. It has been suggested that insulin resistance and decreased adrenergic responsiveness, including attenuated receptor density and postreceptor mechanisms, may contribute to the depressed cardiac contractile function in obesity. Evidence has suggested that the satiety factor secreted by adipose tissue, leptin, may be a link between adiposity and insulin resistance, inasmuch as there is a close association between hyperleptinemia and hyperinsulinemia.

Leptin has been demonstrated to induce proliferation, differentiation, and functional activation of hemopoietic and embryonic cells. Therefore, one could hypothesize that leptin might also play a role in the functional activation of the cell at the myocardial level. Administration of leptin has been shown to increase renal, adrenal, and lumbar sympathetic nerve activity. However, this generalized sympathoexcitatory activity is not always followed by an increase in arterial pressure. This has been credited, to a certain extent, to the possibility that the leptin-induced release of NO may contribute to the homeostasis of the cardiovascular system. The fact that L-NAME inhibition unmasked a positive response of leptin (100 nmol/L) on intracellular Ca

\(^{2+}\) transients, observed in the present study, may support the notion of a direct sympathetic effect by leptin. Ambient NO levels have been shown to modulate cardiac contractile function. Constitutive and inducible NOS are present in cardiac myocytes. The fact that NO donor SNAP exerted a similar depressive effect on cardiac contraction, although to a larger extent because of the overwhelming NO production, added further support to this leptin-induced cardiac response. Because the levels of circulating leptin are increased in all types of obesity, except in the ob/ob mice, the depressed cardiac contractility seen in obesity may be due, in part, to the presence of excessive circulating levels of leptin.

One important defect of cardiac contraction in obesity is the decrease in diastolic compliance and prolonged relaxation. This prolongation may be related to the ventricular hypertrophy–induced reduction of sarcoplasmic Ca

\(^{2+}\) transients, observed in the present study, may support the notion of a direct sympathetic effect by leptin. Ambient NO levels have been shown to modulate cardiac contractile function. Constitutive and inducible NOS are present in cardiac myocytes. The data in the present study indicate that leptin increases NOS activity in cardiac myocytes. This may lead directly to depression of cardiac cell contraction. Further study is warranted to determine the involvement of specific isoforms of constitutive NOS (neuronal or endothelial NOS) in leptin-induced cardiac response.

There has been some debate regarding the role of leptin in cardiac disease. Serum leptin levels may be different according to the clinical stage of the heart problem, ie, an early- to mid-stage increase and an end-stage decline, specifically in patients with cachexia. Multiple factors have been implicated in the regulation of leptin, many of which are subject to endocrine and metabolic influences themselves. The increased leptin levels in early-stage cardiac problems and decreased levels in cachectic end-stage problems may be related to the predominant decline of muscle mass during the initial phases of the disease, with subsequent reduction of the lean/fat ratio. The reduction in this ratio directly promotes leptin production. The decline in leptin levels that occurs in obesity, except in the ob/ob mice, 7,23–25 the depressed cardiac contractility seen in obesity may be due, in part, to the presence of excessive circulating levels of leptin.

**Figure 3.** A. Concentration-dependent response of leptin (0.1 to 1000 nmol/L) on NOS activity in myocytes from adult rat hearts. Incubation time was 60 minutes. B. Time-dependent response of leptin (10 nmol/L) on NOS activity. Values are mean ± SEM (n=4 to 6 assays). *P<0.05 vs control value.

- **Figure 4.** Effect of NO donor SNAP on myocyte shortening in ventricular myocyte isolated from adult male rat heart. Myocyte shortening and relengthening were recorded before and 15 minutes after SNAP (10 to 100 μmol/L) administration. Values are mean ± SEM (n=10 cells/group). *P<0.05 vs baseline.
cachexia with advanced disease may be due to an additional decline in adipose tissue mass accompanied by the loss of body weight. Whether abnormal leptin or its receptor is implicated in the pathogenic process of cardiac diseases or, more likely, is a result of the cardiac and metabolic derangement needs to be further clarified.

In conclusion, the present study demonstrates, for the first time, the cardiac depressive action of leptin. Although these data provide a small step toward elucidating the role of leptin in obesity-related cardiac dysfunction, the role of leptin in mediating the autonomic, cardiovascular, renal, and endocrine changes associated with increased adiposity is still unclear and deserves further study.

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