Leptin-induced suppression of cardiomyocyte contraction is amplified by ceramide

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1. Introduction

Leptin, the product of the obese ob gene, is a peptide hormone mainly found in adipose tissue [28]. Leptin exerts a wide variety of biological actions including inhibition of food intake, promotion of energy expenditure and regulation of cardiovascular function [5,12,16,27]. Leptin may participate in the regulation of cardiac function through sympathetic stimulation and its myogenic inhibition of cardiomyocyte contraction [12,15,16], and has been speculated to contribute to obesity-related cardiac contractile dysfunction [11,16,27]. The levels of leptin in circulation are reported to be significantly elevated under insulin-resistant states such as obesity, overfeeding and hypertension [4,9,10,14,16,26,27]. Furthermore, plasma leptin levels are closely correlated with insulin resistance and increased myocardial wall thickness, independent of body composition and blood pressure levels [6,13]. However, the precise mechanism of action involved in leptin-induced cardiac contractile dysfunction in hyperleptinemic conditions such as obesity is still lacking. Since

Abstract

Uncorrected obesity is often accompanied by ventricular contractile dysfunction, elevation of the lipotoxic mediator ceramide and the obesity gene product leptin. Both ceramide and leptin participate in the regulation of cardiac function and are speculated to play roles in obesity-related cardiac dysfunctions. The purpose of this study was to examine the effect of ceramide on leptin-elicited cardiac contractile response. Adult rat left ventricular myocytes were incubated for 24 h with low (5 nM) or high (50 nM) concentration of leptin in the absence or presence of the active ceramide analog C2-dihydroceramide (25 μM). Contractile and intracellular Ca2+ properties were evaluated using an IonOptix MyoCam® system including peak shortening (PS), maximal velocity of shortening/relengthening (±dL/dt), time-to-PS (TPS), time-to-90% relengthening (TR90), intracellular Ca2+ rise (Δ[Ca2+]i) and intracellular Ca2+ decay. While ceramide did not elicit any effect on cell mechanics and intracellular Ca2+ transients, it sensitized leptin-induced effects on myocyte shortening and intracellular Ca2+ transients. In the absence of ceramide, 5 nM leptin had no effect on cell mechanics while 50 nM depressed PS, ±dL/dt, Δ[Ca2+]i and prolonged TR90. With ceramide co-incubation, 5 nM leptin depressed PS, ±dL/dt, Δ[Ca2+]i and prolonged TR90 whereas 50 nM leptin-elicited effects on PS, ±dL/dt, Δ[Ca2+]i and TR90 were significantly potentiated in addition to slowing intracellular Ca2+ decay. In summary, our data demonstrated that ceramide sensitizes cardiac depressive effects of leptin and may contribute to hyperleptinemia-related cardiac contractile dysfunction.

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obesity is a lipotoxic disease with dyslipidemia including overtly elevated ceramide levels, the de novo ceramide pathway has been postulated to be key to the lipoapoptosis of pancreatic β-cells and cardiomyocytes in obese individuals [24,29]. Nevertheless, the direct effect of ceramide on metabolic and other biological actions of leptin has not been elucidated. The purpose of this study was to examine the effect of short-term ceramide incubation on low (5 mM) and high (50 nM) leptin-induced cardiac contractile response and intracellular Ca2+ handling properties using a serum-free cardiomyocyte primary culture model established in our laboratory [17].

2. Materials and methods

2.1. Isolation of adult rat ventricular myocytes and primary cell culture

The experimental procedures were approved by the animal investigation committees of the University of North Dakota (Grand Forks, ND) and University of Wyoming (Laramie, WY). Single ventricular myocytes were isolated from adult male Sprague–Dawley rats (200–225 g) as described previously [17]. In brief, animals were sacrificed under ketamine/xylazine sedation (5:3, 1.32 mg/kg, i.p.). Hearts were removed and perfused (at 37 °C) with oxygenated (5% CO2–95% O2) Krebs–Henseleit bicarbonate (KHB) buffer (NaCl 118 mM, KCl 4.7 mM, CaCl2 1.25 mM, MgSO4 1.2 mM, KH2PO4 1.2 mM, NaHCO3 25 mM, N-[2-hydro-ethyl]-piperazine-N′-[2-ethanesulfonic acid] (HEPES) 10 mM, glucose 11.1 mM, pH 7.4). Hearts were subsequently perfused with a nominally Ca2+-free KHB buffer containing BSA (2 mg/ml), L-carnitine (2 mM), creatine (5 mM), taurine (5 mM), glucose (5 mM), insulin (0.1 µM), penicillin (100 U/ml), streptomycin (100 mg/ml) and gentamicin (100 mg/ml). Myocytes were incubated at 37 °C in a serum-free medium consisting of Medium 199 (Sigma) with Henseleit bicarbonate (KHB) buffer (NaCl 118 mM, KCl 4 mM, CaCl2 1 mM, MgCl2 1 mM, glucose 10 mM, HEPES 10 mM, at pH 7.4). The cells were field stimulated at a frequency of 0.5 Hz, 3 ms in duration. A video-based edge-detector was used to capture and convert changes in cell length during shortening and relengthening into an analog voltage signal. Cell shortening and relengthening were assessed using the following indices: peak twitch amplitude (PS), time-to-90% PS (TPS) and time-to-90% relengthening (TR90), maximal velocities of shortening (+dL/dt) and relengthening (−dL/dt), respectively.

2.2. Cell shortening/relengthening measurements

Mechanical properties of ventricular myocytes were assessed using an IonOptix MyoCam® system (IonOptix, Milton, MA) as described previously [17]. In brief, coverslips with cells attached were placed in a chamber mounted on the stage of an inverted microscope (Olympus X-70) and superfused (~2 ml/min at 25 °C) with a buffer containing: NaCl 131 mM, KCl 4 mM, CaCl2 1 mM, MgCl2 1 mM, glucose 10 mM, HEPES 10 mM, at pH 7.4. The cells were field stimulated at a frequency of 0.5 Hz, 3 ms in duration. A video-based edge-detector was used to capture and convert changes in cell length during shortening and relengthening into an analog voltage signal. Cell shortening and relengthening were assessed using the following indices: peak twitch amplitude (PS), time-to-90% PS (TPS) and time-to-90% relengthening (TR90), maximal velocities of shortening (+dL/dt) and relengthening (−dL/dt), respectively.

2.3. Intracellular Ca2+ transient measurement

For these experiments myocytes were loaded with fura-2/AM (0.5 µM) for 10 min at 25 °C. Fluorescence measurements were recorded with a dual-excitation single-emission fluorescence photomultiplier system (IonOptix). Myocytes were placed on an inverted microscope and imaged through an Olympus Fluor 40 oil objective. Myocytes were exposed to light emitted by a 75 W halogen lamp through either a 360 or 380 nm filter while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480 and 520 nm by a photomultiplier tube after initial illumination at 360 nm for 0.5 s and then at 380 nm for the duration of the recording protocol. The 360 nm excitation scan was repeated at the end of the protocol and qualitative changes in intracellular Ca2+ concentration ([Ca2+]i) were inferred from the ratio of fluorescence intensity obtained at both wavelengths. Intracellular Ca2+ decay time was calculated from the fluorescence decay curve as an indicator for intracellular Ca2+ clearing.

2.4. Data analysis

Data were expressed as mean ± S.E.M. Statistical comparisons were performed by analysis of variance (ANOVA) followed by Newman–Keuls post hoc test. p < 0.05 was considered statistically significant.

3. Results

As shown in Fig. 1, 24 h incubation of leptin (5 nM) with cardiac myocytes did not exhibit any obvious effect on cell shortening. However, higher concentration of leptin (50 nM) significantly depressed peak shortening (PS) amplitude, maximal velocities of shortening/relengthening (+dL/dt) and prolonged duration of relengthening (TR90) without affecting duration of shortening (TPS). Twenty-four hours incubation of C2-ceramide (25 µM) itself did not exhibit any effect on cell shortening mechanics, consistent with our previous finding [3]. Interestingly, C2-ceramide significantly potentiated leptin-induced cardiac contractile response. In the presence of C2-ceramide, low concen-
tration leptin (5 nM) significantly suppressed peak shortening amplitude, \( \pm \Delta L/dt \) and prolonged TR90 without affecting TPS. The high concentration leptin (50 nM)-induced depression of peak shortening, \( \Delta \left[ Ca^{2+} \right] \) and prolongation of TR90 was further potentiated by C2-ceramide co-incubation. In addition, 50 nM leptin significantly shortened TPS in the presence of C2-ceramide, the effect of which was not seen in the absence of C2-ceramide. To determine whether C2-ceramide-induced potentiation of leptin-induced cardiac contractile response was due to reduced availability of intracellular-free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]), we used fura-2 to evaluate changes in intracellular Ca\(^{2+}\) levels in response to electrical stimuli. The time course of fluorescence signal decay was calculated to assess intracellular Ca\(^{2+}\) clearing rate. Similar to our observation in cell shortening, 5 nM leptin did not affect baseline [Ca\(^{2+}\)], peak [Ca\(^{2+}\)], changes of intracellular Ca\(^{2+}\) (\( \Delta \left[ Ca^{2+} \right] \)) and intracellular Ca\(^{2+}\) decay rate, whereas 50 nM leptin significantly reduced baseline [Ca\(^{2+}\)], peak [Ca\(^{2+}\)], \( \Delta \left[ Ca^{2+} \right] \) without affecting intracellular Ca\(^{2+}\) decay rate (Fig. 2). Interestingly, although ceramide itself failed to affect intracellular Ca\(^{2+}\) handling properties, it significantly sensitized leptin-induced response on intracellular Ca\(^{2+}\) transients. In the presence of C2-ceramide, low concentration leptin (5 nM) significantly suppressed \( \Delta \left[ Ca^{2+} \right] \) without affecting the baseline or peak [Ca\(^{2+}\)] as well as intracellular Ca\(^{2+}\) decay rate. Although the high concentration leptin (50 nM)-induced depression of baseline and peak [Ca\(^{2+}\)] was not affected by C2-ceramide, depression of \( \Delta \left[ Ca^{2+} \right] \) elicited by 50 nM leptin was significantly augmented by C2-ceramide. While 50 nM leptin itself failed to affect the intracellular Ca\(^{2+}\) decay rate, it significantly slowed intracellular Ca\(^{2+}\) clearing rate in the presence of C2-ceramide (Fig. 2).

### 4. Discussion

This study demonstrated that 24 h co-incubation of the ob gene product leptin with cardiac myocytes led to depressed cell shortening and intracellular Ca\(^{2+}\) transients at high (50 nM) but not low (5 nM) leptin concentration, consistent with our previous finding [12]. Although ceramide itself failed to elicit any overt effect on cell shortening and intracellular Ca\(^{2+}\) transients following 24 h incubation, it significantly potentiated the cardiac contractile response of the adipokine leptin. As circulating levels of leptin are increased in almost all types of obesity except in ob/ob mice [4,6,7,10] accompanied with elevated cellular ceramide level and de novo synthesis [23–25,29], the depressed cardiac contractility seen in hyperleptinemic obesity [1,2,18,19] may be due, in part, to the synergistic action of the two lipid mediators on cardiomyocyte contractile function.
Leptin possesses a wide variety of biological responses ranging from cell proliferation, differentiation, to functional activation of hemopoietic and embryonic cells [5,20,22]. Recent evidence also depicted a role of leptin in cardiac contractile regulation either directly through cardiomyocyte contraction or indirectly via enhanced sympathetic nerve activity [12,15,16]. Low nanomolar levels of leptin are considered between physiological and pathophysiological range [9,13]. Our current study did not reveal any overt cardiac contractile response for leptin at concentrations below 10 nM, consistent with our earlier observations [12,27]. Therefore, there is a seemingly disconnection between in vivo and in vitro leptin levels and window of regulation for cardiac contractile function. Since obesity is often associated with elevated synthesis and cellular levels of the lipotoxic mediator ceramide [23,25,29], our observation that ceramide may potentiate or augment cardiac depressive effect of leptin may provide an explanation for hyperleptinemia-associated cardiac dysfunction. It is believed that elevated triglyceride and fatty acid levels in overfeeding and obesity stimulate serine palmitoyl transferase activity resulting in ceramide synthesis de novo and elevated ceramide levels [23,25].

Compromised ventricular function is commonly seen in obesity with insulin resistance and decreased adrenergic responsiveness being the most predominant contributing factors [8,21]. Recent evidence has implicated a role of the satiety factor leptin in cardiac contractile dysfunction in obesity characterized by decreased contractility, reduced diastolic compliance and prolonged relaxation [1,2,18,19]. Results from our current study indicated that short-term incubation of leptin with cardiac myocytes reduced peak shortening amplitude, maximal velocity of shortening/relengthening and prolonged duration of relaxation. Perhaps the most important finding is that a low level of leptin, which displays no effect on cardiac contraction by itself, significantly depresses cardiac contractile response in the presence of ceramide. This may put individuals with normal leptin levels but raised ceramide levels at risk of developing heart dysfunction. The higher level (50 nM) leptin-induced cardiac contractile responses were further exacerbated by ceramide. Ceramide also unmasked additional effects of leptin on duration of shortening and intracellular Ca\(^{2+}\) clearing rate. These observations are supported by shortened contraction duration [27] and reduced rate of intracellular Ca\(^{2+}\) clearing [18] reported in cardiomyocytes from hyperleptinemic individuals. It is worthy mentioning that the mechanical effects are supported by the fact of reduced intracellular Ca\(^{2+}\) rise in response to electrical stimuli, suggesting an alteration of intracellular Ca\(^{2+}\) handling is likely responsible for the synergistic effect of ceramide and leptin on the cardiac contractile response in single cardiac myocytes.
In conclusion, our present study demonstrated, for the first time, a synergistic effect between leptin and ceramide on cardiac contractile function and intracellular Ca\(_{2+}\). These data should shed some light on the elucidation of the role of leptin in hyperleptinemia-induced cardiac dysfunction. Further study is warranted to examine the cellular machineries such as sarco(endo)plasmic reticulum Ca\(_{2+}\)-ATPase (SERCA) and Na\(^+\)/Ca\(_{2+}\) exchanger in this culprit synergy between leptin and ceramide.

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