A neutralizing leptin receptor antibody mitigates hypertrophy and hemodynamic dysfunction in the postinfarcted rat heart

Daniel M. Purdham,1 Venkatesh Rajapurohitam,1 Asad Zeidan,1 Cathy Huang,1 Garrett J. Gross,2 and Morris Karmazyn1

1Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada; and 2Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin

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Purdham DM, Rajapurohitam V, Zeidan A, Huang C, Gross GJ, Karmazyn M. A neutralizing leptin receptor antibody mitigates hypertrophy and hemodynamic dysfunction in the postinfarcted rat heart. Am J Physiol Heart Circ Physiol 295: H441–H446, 2008. First published May 9, 2008; doi:10.1152/ajpheart.91537.2007.—The 16 kDa adipokine leptin has been shown to exert direct hypertrophic effects on cultured cardiomyocytes although its role as an endogenous contributor to postinfarction remodeling and heart failure has not been determined. We therefore investigated the effect of leptin receptor blockade in vivo on hemodynamic function and cardiac hypertrophy following coronary artery ligation (CAL). Cardiac function and biochemical parameters were measured in rats subjected to 7 or 28 days of left main CAL in the presence and absence of a leptin receptor antibody. Animals subjected to an identical treatment in which the artery was not tied served as sham-operated controls. CAL produced myocardial hypertrophy, which was most pronounced 28 days postinfarction as demonstrated by increases in both left ventricular weight-to-body weight ratio and atrial natriuretic peptide gene expression, both of which were abrogated by leptin receptor antagonism. Leptin receptor blockade also significantly improved left ventricular systolic function, attenuated the increased left ventricular end-diastolic pressure, and reduced the expression of genes associated with extracellular matrix remodeling 28 days following CAL. In conclusion, the ability of a leptin receptor-neutralizing antibody to improve cardiac function offers evidence that endogenous leptin contributes to cardiac hypertrophy following CAL. The possibility exists that targeting the myocardial leptin receptor represents a viable and novel approach toward attenuating postinfarction remodeling.

Address for reprint requests and other correspondence: M. Karmazyn, Dept. of Physiology and Pharmacology, Univ. of Western Ontario, London, ON, N6A 5C1, Canada (e-mail: morris.karmazyn@schulich.uwo.ca).

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MATERIALS AND METHODS

Animals and surgical procedures. Adult male Sprague-Dawley rats, weighing 225 to 250 g, were purchased from Charles River Canada (St. Constant, QC, Canada), fed standard rat chow, and maintained in the Health Science Animal Care facility of the University of Western Ontario in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada), and the investigation conforms with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). The protocols used for these studies were reviewed and approved by the Animal Use Subcommittee of the University of Western Ontario.

Experimental design. Rats were randomly assigned to one of five treatment groups: 1) sham, 2) sham + OBR antibody (OBR-Ab), 3) CAL, 4) CAL + OBR-Ab, and 5) CAL + IgG antibody. On the morning of the experiment, rats were anesthetized by an intraperitoneal injection of pentobarbital sodium, intubated, and artificially ventilated using a rodent respirator (model 683, Harvard Apparatus). A left thoracotomy was performed, and the heart was gently excised. To induce myocardial infarction, the left main coronary artery was occluded.
ligated ~3 mm from its origin using a firmly tied silk suture (5-0). For sham-operated surgery, the ligature was placed in an identical fashion but not tied. The chest was then closed in three layers (ribs, muscle, and skin), and the animal was allowed to recover.

The OBR or IgG antibody (Alpha Diagnostic International, San Antonio, TX) (or saline as a vehicle control) was administered immediately following CAL (4 µg/kg body wt) and then every other day until euthanasia either 7 or 28 days postoperatively. Animals in the 7-day group were euthanized by decapitation, and the heart was rapidly excised, weighed, and stored for biochemical and molecular analyses. Rats maintained for the full 28-day follow-up period initially underwent hemodynamic assessment as described below before euthanasia and harvesting of tissue for analyses. Postligation mortality rates of 23% and 27% were observed in control and OBR-Ab-treated rats, respectively, all of which occurred within 24 h after CAL.

**Determination of OBR-Ab efficacy.** To confirm the ability of the OBR-Ab to block the effect of exogenous leptin, rats were fasted overnight and injected the next morning via the saphenous vein under pentobarbital sodium anesthesia with either 1 mg/kg leptin or equal volumes of saline. Rats were euthanized, and left ventricular (LV) tissue was processed for RNA extraction and reverse transcription, followed by real-time PCR for gene expression of leptin, OBRa, OBRb, OBRe, atrial natriuretic peptide (ANP), collagen I, collagen III, and fibronectin. Primers used in real-time PCR analysis can be found in table 1. Primers used in real-time PCR analysis can be found in table 1. Tissue processing and PCR conditions were identical to those previously published (12).

**Infarct size determination.** Studies were done to determine whether any affect of the OBR antibody on chronic responses could be explained by early modification of the infarct size in hearts subjected to acute ischemia and reperfusion. For these experiments, surgical preparation and infarct size determinations were performed as previously described (10).

**Gene expression determination.** Noninfarcted LV tissue was processed for RNA extraction and reverse transcription, followed by real-time PCR for gene expression of leptin, OBRa, OBRb, OBRe, atrial natriuretic peptide (ANP), collagen I, collagen III, and fibronectin. Primers used in real-time PCR analysis can be found in table 1. Tissue processing and PCR conditions were identical to those previously published (12).

<table>
<thead>
<tr>
<th>Gene Primer Annealing Temperature, °C Accession Number</th>
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<tr>
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<tr>
<td>18S Forward 5'-GAACCACTCAGTTGACCAGGCAAG-3' 54 X01117</td>
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<td>Collagen III Reverse 5'-CATTCAGGGCTAAGGTCCAA-3'</td>
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ANP, atrial natriuretic peptide; OBR, leptin receptor.
Data were expressed as means ± SE. All data were analyzed using GraphPad Prism v. 4.0. Comparison between groups was performed using a two-way analysis of variance with post hoc analysis by Bonferroni’s test. A probability value of $P < 0.05$ was considered statistically significant.

**RESULTS**

**Body weights.** Rats treated with OBR-Ab gained significantly more weight than control animals. At the end of the 28-day follow-up period, weight gains for sham-operated and CAL animals treated with the OBR-Ab were 151 ± 10 and 164 ± 7 g, respectively, whereas the respective values for untreated sham-operated and CAL animals were 131 ± 7 and 126 ± 7 g, respectively ($P < 0.05$). CAL had no effect on weight gain irrespective of OBR-Ab treatment (Fig. 1).

**Effectiveness of OBR-Ab in blocking cardiac leptin receptors.** In three separate experiments, leptin increased cardiac STAT phosphorylation, which was completely abrogated in rats pretreated with the OBR-Ab (Fig. 2). These results demonstrated the substantial efficacy in terms of the ability of the OBR-Ab to block the cardiac effects of leptin.

**Plasma leptin concentrations and cardiac leptin receptor expression.** We determined whether CAL altered either the expression of OBR in cardiac tissue or the plasma leptin concentrations. We also determined the expression of leptin in cardiac tissue. Cardiac OBR expression was unchanged 7 days after CAL, although after 28 days, the expression of both OB-Rb and OB-Re was significantly increased in both the sham-operated and CAL animals treated with OBR-Ab. CAL had no direct effect on cardiac OBR expression (data not shown). Cardiac OB-Ra expression levels were unaltered by OBR-Ab treatment or CAL. Plasma leptin concentrations in sham-operated rats averaged $12.90 ± 1.88$ ng/ml, which was not significantly affected by CAL ($17.02 ± 2.70$ ng/ml). Similarly, OBR-Ab treatment had no effect on plasma leptin concentrations in either the sham-operated or CAL groups where plasma leptin concentrations were $15.40 ± 3.57$ and $18.40 ± 3.46$ ng/ml, respectively (data not shown).

**Indexes of cardiac hypertrophy.** We assessed cardiac hypertrophy by determining LV weight-to-body weight ratios (LVW/BW) ratios, as well as gene expression of ANP at both 7 and 28 days after infarction. At 7 days, LVW/BW was unchanged, although a significant fivefold increase in ANP expression was seen, which was significantly attenuated by OBR-Ab treatment (Fig. 3). At 28 days, CAL significantly increased LVW/BW (sham, 1.83 ± 0.04; and CAL, 2.13 ± 0.06 mg/g), which was completely abrogated by OBR-Ab treatment (Fig. 3A). ANP expression was increased 2.5-fold but not in OBR-Ab treated rats (Fig. 3B).

**Hemodynamic function.** LV function was assessed at the end of the 28-day postinfarction period. The OBR-Ab had no direct effect on any parameter in sham-operated animals not subjected to CAL. IgG antibody treatment had no effect in CAL rats compared with saline controls. As shown in Fig. 2, in terms of diastolic dysfunction, LV end-diastolic pressure increased from $1.6 ± 1.1$ mmHg in sham-operated animals to $7.9 ± 1.6$ mmHg in animals subjected to CAL, although the degree of elevation in LV end-diastolic pressure was significantly reduced in animals treated with the OBR-Ab from $2.5 ± 0.7$ mmHg in sham-operated animals to $5.3 ± 0.5$ mmHg in animals treated with the OBR-Ab. Moreover, the 23% reduction in the rate of LV pressure fall was abrogated by the

![Fig. 2](http://ajpheart.physiology.org/content/295/1/H444/F2)

![Fig. 3](http://ajpheart.physiology.org/content/295/1/H444/F3)

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OBR-Ab, as were the significant decreases in LVDP and rate of LV pressure development (Fig. 4).

**Extracellular matrix remodeling.** The expression of fibronectin, as well as the two primary collagen isoforms, type I and type III, were studied at 7 and 28 days postligation. As shown in Fig. 5, the expression of fibronectin and types I and III collagen were significantly increased 7, but not 28, days postligation. However, the elevation seen at day 7 was prevented by the OBR-Ab treatment.

**Infarct size determination.** To determine whether the salutary effects of the OBR-Ab on postinfarction remodeling and hemodynamics reflected a myocardial salvaging property of the antibody, experiments were done to determine the effect of the antibody on infarct size in animals subjected to acute coronary ligation and reperfusion. However, no effect on infarct size was observed with virtually identical infarct sizes (expressed as percentage of the area at risk) for the two CAL groups (control, 62.2 ± 1.6%; and OBR-Ab, 62.2 ± 2.2%).

**DISCUSSION**

The obesity-related satiety factor leptin has been proposed as a possible biochemical link between obesity and increased propensity for cardiovascular disorders (8). The ability of cardiac tissue to synthesize leptin (12), as well as the identification of OBR in cardiomyocytes (12, 13), is suggestive of a direct effect of leptin acting in an autocrine or paracrine manner. In terms of cardiac pathology, the most widely studied phenomenon vis-à-vis the effects of leptin is the ability of the polypeptide to produce hypertrophy in primary cardiomyocyte cultures of both rats (13, 19, 20) and humans (6). However, whether leptin exerts hypertrophic effects in vivo has not been extensively studied. Indeed, in obese ob/ob mice, leptin has been shown to reduce cardiac hypertrophy; however, the reversal of hypertrophy by leptin was associated with normalization of body weight, thus rendering the establishment of a cause-and-effect relationship difficult (1). In the present study we reasoned that the blockade of leptin receptors may more precisely delineate a role of endogenous leptin in cardiac pathology. We concentrated our study on cardiac hypertrophy and ventricular function based on a clinical association between hyperleptinemia and heart failure, independently of obesity (15), as well as an increasing number of studies demonstrating a direct

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*Fig. 4. Intraventricular pressures in vivo 28 days after CAL. Pressures were obtained using a Millar catheter pressure transducer inserted into the LV. CAL increased LV end-diastolic pressure (LVEDP; A) and decreased all other parameters in control rats but not in the presence of OBR-Ab. B: LV developed pressure (LVDP). C and D: rates of LV pressure development (+dP/dt; C) and fall (−dP/dt; D). Values shown are means ± SE; n = 12 animals. *P < 0.05 vs. sham Ctrl; **P < 0.01 vs. sham Ctrl.*

*Fig. 5. Expression of fibronectin and collagen isoforms 7 and 28 days post-CAL. Fibronectin as well as both collagen isoforms were significantly increased at 7 days with no differences seen 28 days after CAL. All increases in expression were prevented in animals treated with the OBR-Ab. Values shown are means ± SE; n = 12 animals. *P < 0.05 vs. sham Ctrl; **P < 0.01 vs. sham Ctrl; +P < 0.05 vs. CAL Ctrl.*
hypertrophic effect of leptin. The major finding of our study is that a leptin receptor-neutralizing antibody significantly improved the myocardial response to sustained coronary artery occlusion as manifested by ameliorated hemodynamics and reduced hypertrophy, as well as diminished expression of factors involved in extracellular remodeling.

The dose of antibody used in this study was sufficient in terms of its ability to block OBR (the antibody is not specific for any isoform of OBR), in view of increased body weights observed in all rats treated with the OBR-Ab, as well as the ability of the OBR-Ab to block the leptin-induced increase in cardiac STAT phosphorylation. Taken in concert with the lack of effect of IgG antibody treatment on hemodynamic parameters following CAL, it is likely that the effect of the OBR-Ab seen in our study was due to OBR blockade. Taken together, our findings imply that endogenously synthesized leptin contributes to the remodeling process in the absence of obesity.

Four weeks of CAL did not affect plasma leptin concentrations. This differs from clinical studies where heart failure is associated with hyperleptinemia (5, 15), and indeed the latter has been proposed as a potential diagnostic tool for identifying heart failure in patients (16). Since the degree of hyperleptinemia may be related to the severity of heart failure or hypertrophy (15), this may explain the inability to observe a significantly increased level of plasma leptin in view of the moderate hypertrophy and LV dysfunction seen 4 wk postligation in our study.

A key finding in this study was the preservation of LV function 28 days post-CAL in rats treated with OBR-Ab, implicating endogenous leptin as a contributor to cardiac dysfunction. It is likely that at least part of the salutary effect of the OBR-Ab was due to the inhibition of the prohypertrophic effect of leptin as shown in numerous studies using cultured cardiomyocytes (6, 13, 18). Thus the improved LV function and reduced hypertrophy in vivo likely reflected a blockade of leptin-hypertrophic influence that was manifested by reduced indexes of LVW/BW and ANP expression. Another factor that could contribute to improve LV function is reduced extracellular remodeling, and, accordingly, we studied the expression levels of various components important for remodeling at both early (7 days) and late (28 days) postinfarction. In our study, fibronectin expression was elevated 7 days following ligation, but not at 28 days, with a similar profile observed for both the collagen-I and collagen-III isoforms. The ratio of collagen isoforms is an important determinant of myocardial stiffness, and it is thought that an increase in type I-to-type III collagen ratio results in a stiffening of the myocardium (2). Importantly, the early increase in expression of both fibronectin and collagen expression was almost completely abrogated by the OBR-Ab. The early increase in fibronectin and collagen isoforms is consistent with an imbalance of profibrotic to degradation molecules that would lead to cardiac stiffening.

Although the present study was not designed to examine cellular mechanisms underlying the antihypertrophic effects of OBR blockade, our results support and complement previous findings from our laboratory that have demonstrated a prohypertrophic effect of leptin in cultured ventricular myocytes (13, 14, 19, 20). Moreover, we have shown that leptin-induced hypertrophy is mediated by the activation of the RhoA/Rho-associated coiled-coil-containing kinase (ROCK) pathway resulting in enhanced actin polymerization (19). This effect is dependent on intact caveolae and results in selective p38 MAPK translocation into nuclei (20). Thus Rho/ROCK signaling, resulting in changes in actin dynamics and induction of p38 nuclear translocation, likely represents a major intracellular pathway important for mediating the hypertrophic effects of leptin although it remains to be determined whether this pathway can be implicated in mediating postinfarction remodeling in vivo.

In conclusion, our study shows for the first time that endogenous leptin contributes to postinfarction responses in rats subjected to 4 wk of sustained CAL. The beneficial effects of leptin receptor-neutralizing antibody was manifested as improved LV function and reduced ventricular hypertrophy. Intriguingly, the effects of the OBR antibody occurred in nonobese animals exhibiting a modest but not significant increase in plasma leptin concentrations following CAL. When taken together, this would suggest that an obesity-induced hyperleptinemic state is not a prerequisite for implicating leptin as a contributor to postinfarction remodeling. Although extrapolation of these findings to human pathology should be done cautiously, the findings suggest that endogenously synthesized leptin may therefore represent one of the many factors involved in the postinfarction remodeling process and a potential target for therapeutic intervention.

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REFERENCES


