The primary binding protein for leptin is a soluble form of the leptin receptor (OB-Re) (12). Six isoforms of OB-R have been identified: OB-Ra, OB-Rb, OB-Rc, OB-Rd, OB-Re, and OB-Rf. Each has a leptin binding domain and transmembrane region (except OB-Re) but differ in the length of the intracellular domain. One receptor isoform, known as the long form (OB-Rb), represents the full signaling isoform of the leptin receptor (4). Collectively known as the short forms, OB-Ra, OB-Rc, OB-Rd, and OB-Rf differ from one another in species and tissue distribution; however, it is unknown whether they mediate altered functional responses. The short forms have signaling capabilities that are generally considered to be less than those of OB-Rb (4). It has been suggested that the short forms act to transport leptin across barriers and/or act as a source of OB-Re through a proteolytic cleavage of the extracellular leptin binding site (2, 11).

Recent evidence suggests that adipocytes may not represent the only source of leptin. For example, leptin expression has been identified in the rat gastric epithelium (1) as well as the placenta (15). On the basis of clinical evidence that leptin may be related to heart disease, we hypothesized that the heart may indeed be a source of leptin production. Moreover, OB-Ra, OB-Rb, and OB-Re have been identified in mouse heart homogenates (12, 14). Leptin has been shown to exert direct effects on cardiomyocytes; these effects include a direct negative inotropic effect on adult rat ventricular myocytes (18) and a direct hypertrophic influence on cultured rat neonatal ventricular myocytes (20). On the basis of these findings, the present study was conducted to determine whether leptin and its receptors are expressed in the rat heart and whether this expression can be modulated by ischemia and without reperfusion. We further investigated regional distribution of the leptin system in the heart and determined whether there are any gender differences in the nature of the cardiac leptin system.

MATERIALS AND METHODS

Animals and perfusion of hearts. Adult male and female Sprague-Dawley rats (225–250 g; Charles River Canada, St. Constant, Quebec, Canada) were 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, Ontario, Canada). Rats were killed by decapitation, and hearts were immediately excised and subjected to retrograde aortic perfusion with Krebs-Henseleit buffer consisting of (in mM) 120 NaCl, 4.63 KCl, 1.17 KH2PO4, 1.2 MgCl2, 1.25 CaCl2, 20 NaHCO3, and 8 glucose, pH 7.4, at a flow rate of 10 ml/min. After all hearts were equilibrated for 30 min, a 30-min ischemic period was induced by complete cessation of flow with and without 30 min of reperfusion. Control hearts were perfused without ischemia for 60 or 90 min, as appropriate.

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Tissue RNA isolation and reverse transcription. At the end of the experimental protocol, hearts were removed from the cannula and dissected into five parts, the right atrium (RA), left atrium (LA), intraventricular septum (IVS), right ventricle (RV), and left ventricle (LV), and placed in TRIzol reagent (Invitrogen, Carlsbad, CA) on ice. Tissues were homogenized and RNA was isolated according to the manufacturer’s instructions. Five micrograms of total RNA were used to synthesize the first strand of cDNA using random hexamer (Invitrogen) and oligo(dT) (Invitrogen) primers and SuperScript II RNase H−reverse transcriptase (Invitrogen) according to the manufacturer’s protocol. The cDNA was diluted 10-fold, and 1 μl of the diluted cDNA was used in a 20-μl PCR vessel.

Myocyte isolation. Myocytes were isolated as previously described (9). Briefly, hearts were mounted on a modified Langendorff apparatus and perfused with Ca2+-free buffer containing (in mM) 120 NaCl, 5.4 KCl, 1 MgCl2, 0.33 NaH2PO4, 10 HEPES, and 10 glucose, pH 7.4. After a brief equilibration period, type II collagenase (1.16 mg/ml; Worthington Biochemical, Lakewood, NJ) and protease type XIV (0.1 mg/ml; Sigma-Aldrich, St. Louis, MO) were added to the buffer, and the heart was perfused for 9 min in a recirculating manner. Collagenase was washed out with buffer containing 0.2 mM Ca2+ and diced with scissors; then the heart was anatomically separated into LV and RV + IVS. After incubation at 37°C, tissues were filtered through a nylon mesh and allowed to settle. Cells were exposed to a series of sedimentation and resuspension steps in buffer containing increasing concentrations of Ca2+ ranging from 0.2 to 1.0 mM. Cells were prepared for RNA isolation as described above.

Real-time PCR analysis of leptin and leptin receptor expression. Real-time PCR was used to analyze the gene expression of leptin and leptin receptor (OB-R) isoforms (OB-Ra, OB-Rb, and OB-Re). 18S rRNA gene expression was also measured as a control. PCR and melting curve analyses were performed in 20-μl reaction volumes using SYBR green JumpStart Taq ReadyMix DNA polymerase (Sigma-Aldrich), and fluorescence was measured using a DNA Engine Opticon 2 system (MJ Research, Waltham, MA). Amplification was performed using the following primers: 5'-GTAACCCGTGTGAAACCCATT-3' (forward) and 5'-CCATCCATGCTGATTGCG-3' (reverse) for 18S rRNA, 5'-GAGACCTCTCCTCATGTGCCG-3' (forward) and 5'-CATCAGGGCTAAGTGTCCAA-3' (reverse) for leptin, 5'-GTGATATGCGCAACAGCGCA-3' (forward) and 5'-AGTGTCCTCTCTTGGAGA-3' (reverse) for OB-Ra, 5'-TGACCCTGGACCTTGCGGAAA-3' (forward) and 5'-CCACTTGTTTACAGTGTCG-3' (reverse) for OB-Rb, and 5'-AAAGTGCTTAAATCCCTTGCG-3' (forward) and 5'-CAGTCTTCTGACACGCAAG-3' (reverse) for OB-Re. PCR conditions used to amplify all five genes were 30 s at 94°C, 55°C for 20 s, and 72°C for 30 s, with the exception of 18S, for which the annealing temperature was 55°C. 18S was amplified over 35 cycles, and leptin and all isoforms of OB-R were amplified for 40 cycles.

Determination of leptin release from isolated hearts. Effluent was collected at selected times from isolated perfused hearts exposed to 30 min of ischemia followed by 30 min of reperfusion. Leptin was measured using a TiterZyme enzyme immunometric assay kit (Assay Designs, Ann Arbor, MI).

Statistical analysis. Values are means ± SE. All data were analyzed using Graphpad Prism version 3.0 and Graphpad InStat version 3.05. Comparison between groups was performed using a Student’s t-test or a one-way analysis of variance with post hoc analysis by Student-Newman-Keuls test. P < 0.05 was considered statistically significant.
In female rats, leptin expression was higher in the RA than in all the other regions (Fig. 3A). Moreover, leptin gene abundance in the RA was approximately eightfold higher in female than in male rats (Fig. 3A). We also determined the ability of isolated hearts to release leptin into the coronary effluent under normal conditions as well as during postischemic reperfusion. Basal leptin levels were 60 and 70 pg/ml in male and female rats, respectively (Fig. 3B). A brief elevation in leptin release was evident immediately on restoration of flow after ischemia (depicted as 60 min). This likely represented posts ischemic washout, inasmuch as leptin release promptly returned to preischemic basal values within 1 min of reperfusion (Fig. 3B).

Relative gene expression patterns of leptin receptors in different cardiac regions are shown in Fig. 4. No differences were seen in male hearts, although levels of expression of all three receptors were significantly higher in the RA than in most other regions in female rat hearts. Additionally, receptor expression was much higher in the RA from female than from male rats. The other notable differences were the significantly higher levels of OB-Ra in the LV from female rats (Fig. 4A) and the significantly higher OB-Re expression in the IVS of male than of female hearts.

We next determined the effect of ischemia with or without reperfusion on leptin gene expression (Fig. 5) as well as expression of OB-Ra, OB-Rb, and OB-Re (Figs. 6–8). With respect to leptin itself, expression was significantly decreased after ischemia (Fig. 5A) and after reperfusion (Fig. 5C) in most regions of the heart from male animals; however, in reperfused hearts, these differences were restricted to the LA and IVS (Fig. 5C). In contrast, hearts from female rats were more

![Fig. 2. Expression of leptin (A), OB-Ra (B), OB-Rb (C), and OB-Re (D) in isolated adult cardiomyocytes from right ventricle (RV; including intraventricular septum (IVS)) and left ventricle (LV). Values are means ± SE (n = 8). *Significantly different from male, P < 0.05. +Significantly different from female right ventricle, P < 0.05.](http://ajpheart.physiology.org/)

![Fig. 3. A: gender and regional distribution of leptin gene expression in tissue homogenates from adult rat hearts. Expression was measured in right atrium (RA), left atrium (LA), IVS, RV, and LV. Values are means ± SE (n = 8). *Significantly different from male, P < 0.05. #Significantly different from all other females, P < 0.05. $Significantly different from all other males, P < 0.05. B: leptin release from isolated perfused heart. Values are means ± SE (n = 6).](http://ajpheart.physiology.org/)
resistant to ischemia-induced changes in leptin expression, although significant decreases in the RA and IVS were seen in reperfused hearts.

In terms of leptin receptors, male rats showed an ischemia-induced decrease in OB-Ra expression in the RA, RV, and LV (Fig. 6A). Female rats also showed a decrease in OB-Ra expression in the RA; however, in contrast to male rats, OB-Ra expression was increased in the RV and LV (Fig. 6B). Postischemia reperfusion produced an upregulation of OB-Ra in the RA of male rats and a decrease in IVS and LV expression (Fig. 6C), whereas female rats exhibited decreased OB-Ra expression levels in the RA, IVS, and LV (Fig. 6D).

Fig. 4. Gender and regional distribution of OB-Ra (A), OB-Rb (B), and OB-Re (C) in tissue homogenates from adult rat hearts. Values are means ± SE (n = 8). Significant differences between respective males and females: *P < 0.05; **P < 0.01. Values with identical superscripts (a, b) are not significantly different from one another.

Fig. 5. Effects of 30 min of ischemia without (A and B) or with (C and D) 30 min of reperfusion on leptin gene expression in various regions of rat heart. Values are means ± SE (n = 8). Significant difference between ischemic and time control groups: *P < 0.05; **P < 0.01.
Fig. 6. Effects of 30 min of ischemia without (A and B) or with (C and D) 30 min of reperfusion on OB-Ra gene expression in various regions of rat heart. Values are means ± SE (n = 8). Significant difference between ischemic and time control groups: *P < 0.05; **P < 0.01.

Fig. 7. Effects of 30 min of ischemia without (A and B) or with (C and D) 30 min of reperfusion on OB-Rb gene expression in various regions of rat heart. Values are means ± SE (n = 8). Significant difference between ischemic and time control groups: *P < 0.05; **P < 0.01.
OB-Rb expression was generally unaffected by ischemia in terms of statistical significance, although a trend toward depressed expression was evident in hearts from male animals (Fig. 7A), whereas a significant reduction in OB-Rb expression was observed in the RV from female hearts (Fig. 7C). Reperfusion produced significant reductions in OB-Rb expression in various regions of the hearts of male and female rats (Fig. 7, B and D).

Expression of the soluble isoform OB-Re was decreased by ischemia in most regions of male hearts (Fig. 8A), whereas in reperfused hearts a decrease in OB-Re expression levels was seen in the IVS and LV (Fig. 8C). Female hearts were unaffected (Fig. 8, B and D).

**DISCUSSION**

Since its initial discovery, leptin has been generally considered as being derived primarily from adipocytes (25), although subsequent studies demonstrated that the peptide can also be derived from nonadipocyte tissues, including the stomach (1) and placenta (15). Recently, leptin production has been identified in the skin, especially after injury, implicating the peptide in the process of wound healing (17). Our study was aimed at determining whether the heart has the ability to produce leptin, especially in view of recent data showing the direct effects of leptin on cardiac function (18) as well as clinical data revealing enhanced leptin levels in patients with heart failure and ischemic heart disease, which appears to occur independently of obesity (5, 13). In view of the emerging evidence implicating the heart as a target of leptin action, we also determined whether cardiac tissues express leptin receptors and whether the expression of leptin or its receptors can be modulated by ischemic conditions with or without subsequent reperfusion. Finally, we considered it of importance to assess whether any differences exist in the intensity of leptin or leptin receptor expression in different regions of the heart.

The major finding of our study is that the rat heart expresses the leptin peptide as well as at least three of its receptors, including OB-Ra, OB-Rb, and OB-Re. To our knowledge, this is the first report to identify the heart from male and female animals as a source of leptin, thereby strongly suggesting that leptin can modulate cardiac function in an autocrine-dependent manner. Although hearts from male and female rats expressed leptin, it is interesting that, from a general perspective, gene abundance was higher in tissues from female rats. This was especially evident in the RA with respect to all three receptor subtypes, where gene expression (e.g., for OB-Rb) was ~10-fold higher in female than in male rats. In addition, differences in leptin expression were also observed, with 3-fold greater levels of leptin expression in RV myocytes from female than from male rats and a ~10-fold greater RA leptin expression in hearts from female animals. Thus clear quantitative differences exist in the levels of leptin and leptin receptor expression, with markedly higher gene abundance in female hearts, a finding particularly evident, but not completely restricted, to RA tissue.

Because of a potential link between coronary heart disease and elevated leptin levels in patients, we considered it
of value to determine whether myocardial ischemia and subsequent reperfusion affect the expression of the leptin system in terms of leptin release by the heart as well as leptin receptor expression. With respect to the former, our studies with isolated perfused rat hearts show a steady level of leptin efflux, which was generally unaffected by postischemic reperfusion, with the exception of an initial transient burst of leptin efflux immediately at the onset of reperfusion. We believe that this likely represented a postischemic washout phenomenon or a rapid release of leptin from necrotic cells, especially because sustained leptin efflux during reperfusion was not observed. Thus it appears that leptin efflux is unaffected by ischemia and reperfusion, although it remains to be determined whether increasing ischemia severity or duration modulates this response. We utilized a modestly severe ischemic protocol that resulted in an ~50% recovery in function, and it is conceivable that increasing the severity of injury could produce a correspondingly greater leptin efflux profile. The source of leptin in the coronary effluent cannot be confirmed with certainty, although preliminary studies in our laboratory demonstrated that leptin could be detected in serum-free media containing cultured neonatal rat ventricular myocytes (unpublished observations). Thus it appears that a steady-state level of leptin production and release likely occurs from the cardiac cell.

In contrast to the lack of effect of ischemia and reperfusion on leptin efflux, our study shows for the first time that ischemia induces a clear downregulation of leptin and leptin receptor gene expression in isolated hearts, which was overall more prominent in hearts of male than female animals. The relevance of the downregulation of the leptin system in the ischemic myocardium is uncertain and requires further studies. Plasma leptin levels have been shown to be increased twofold in patients with acute myocardial infarction, with peak elevations observed 48 h after hospital admission, and it has been suggested that leptin may contribute to cardiac damage in myocardial infarction patients (16). Whether hyperleptinemia (plasma leptin levels >15 ng/ml) is a risk factor for the development of heart disease has not been resolved. In a study involving French Canadian men, no association between elevated leptin and coronary heart disease could be identified (8). However, a recent large Scottish prospective study identified leptin as an independent risk factor for coronary heart disease in men (23). In addition, a Swedish study recently suggested that elevated leptin levels represent an effective predictor of first-ever myocardial infarction (21). Thus much work needs to be undertaken to appreciate the role of leptin in ischemic heart disease. However, it is intriguing to speculate that downregulation of expression of leptin and its receptors represents an adaptive and protective mechanism under myocardial ischemic conditions. An interesting feature of this hypothesis is the ischemia-induced downregulation of OB-Re, the leptin soluble receptor. This could represent an adaptive response to increase the rate of clearance of leptin from cardiac tissue, because by decreasing OB-Re, while increasing free leptin levels, the rate of leptin elimination would also be stimulated (10).

**Limitations and potential significance of this study.** Our study was designed to address whether the heart could represent a source of leptin and whether it expresses leptin receptors. If so, this would suggest that leptin may serve as an endogenous paracrine or autocrine regulator of cardiac function as are other peptides such as angiotensin II and atrial natriuretic peptide. It was beyond the scope of our study, however, to assess in detail the functional role of leptin in the heart. However, recent evidence suggests that the heart is indeed a target for leptin’s actions. First, leptin has recently been shown to exert a negative inotropic effect on isolated cardiomyocytes through a mechanism potentially involving nitric oxide generation (18). Second, a recent clinical study revealed a significant positive correlation between plasma leptin levels and heart rate in heart transplant recipients, thereby suggesting that leptin directly modulates heart rate (24). Whether high leptin expression in RA mediates chronotropic effects remains to be determined, as does the degree of leptin and leptin receptor distribution in pacemaker cells. Third, further recent evidence suggests that leptin may directly modulate hypertrophic responses in the heart, although whether the peptide is an anti- or a prohypertrophic factor remains to be determined. In this regard, we recently demonstrated a direct hypertrophic effect of the peptide on cultured neonatal rat ventricular myocytes acting via the p38 MAPK system (20), whereas others have shown that leptin can stimulate proliferation of cardiac-derived HL-1 cells (22). Both of these findings thus suggest a progrowth effect of the peptide. Barouch et al. (3), on the other hand, demonstrated ventricular hypertrophy in leptin-deficient mice that was reversed by leptin administration, suggesting an antihypertrophic effect of the peptide. Although these apparent discordant results need to be resolved, taken together, they provide convincing evidence that the heart is an important target for leptin’s effects.

In summary, we have shown for the first time that the heart, including the cardiomyocytes, expresses all the components of the leptin system and also that there are cardiac regional and gender differences in leptin and leptin receptor profiles. Research into cardiovascular aspects of leptin is still in its relative infancy, and the significance of our findings, especially in terms of regional and gender-dependent expression profiles, needs to be fully elucidated with extensive studies, as does the role of leptin in the ischemic myocardium. However, when taken together, our results suggest that the heart is a target for leptin’s actions, and leptin may represent an autocrine/paracrine regulator of cardiac function.

**GRANTS**

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