Endothelin-1 Stimulates Leptin Production in Adipocytes

Received for publication, April 19, 2001, and in revised form, May 17, 2001
Published, JBC Papers in Press, May 18, 2001, DOI 10.1074/jbc.M103478200

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Leptin is an adipocyte-derived hormone that regulates body fat stores and feeding behavior. In an effort to identify endogenous diffusible modulators of leptin production, we found that endothelin-1 (ET-1) up-regulates leptin expression in adipocytes. ET-1 is as potent and efficacious as insulin in stimulating leptin production in two different adipocyte cell lines. Endothelins stimulate leptin production via the endothelin-A receptor (ET_A), as judged by a potency rank order of ET-1 > ET-3. We detected expression of ET_A but not ET_B in both cell lines by Northern blot analysis. In addition, the ET_A-selective antagonist FR139317 inhibited ET-1-induced leptin expression more potently than did the ET_B-selective antagonist BQ788. ET-1 and insulin positively interact with each other in increasing leptin production in adipocytes. In primary mouse white fat cells, we detected expression of both ET_A and ET_B by Northern blot and in situ hybridization analyses. We conclude that ET-1 stimulates leptin production via the ET_A receptor in cultured adipocytes.

The ob³ (obese) gene product, leptin, is a 16-kDa protein secreted predominantly by adipocytes. Administration of recombinant leptin reduces food intake and increases thermogenesis and physical activity in ob/ob mice, resulting in reduced body weight in these mice (1–3). This fat-derived hormone regulates energy homeostasis through leptin receptors expressed in the central nervous system, particularly in the hypothalamus (4). It is also reported that leptin may acutely stimulate glucose metabolism by acting on peripherally expressed leptin receptors (5). Leptin receptor-deficient db/db mice have a similar phenotype as ob/ob mice, including obesity, diabetes, and infertility in females (6, 7). Mutations in either leptin (8) or leptin receptor (9, 10) genes are associated with early-onset obesity in humans. Recent studies demonstrate that leptin is a multifunctional hormone involved in not only energy homeostasis but also onset of puberty (11, 12), sympathetic nerve activity (13), hematopoiesis (14, 15), angiogenesis (16), and immune response (17). In addition to fat, stomach (18), placenta (19), and muscle (20) tissues are minor sources of leptin, suggesting possible undiscovered functions.

Serum leptin levels are elevated in most obese individuals in accordance with their body adiposity (21). Feeding state (22, 23) also regulates leptin expression. These findings indicate that leptin expression is regulated through multiple central and peripheral mechanisms. The finding that subdermally implanted 3T3-F442A preadipocytes gave rise to fat pads and expressed much higher levels of leptin than cells differentiated in vitro also strongly argued for the existence of in vivo regulators of leptin gene expression (24). A number of factors regulate leptin expression in vitro or in vivo. Insulin (23, 25), glucocorticoids (26, 27), and some cytokines (28) are activators of leptin production. In contrast, catecholamine (26, 29) and peroxisome proliferator-activated receptor-γ agonists (30) suppress leptin expression in cultured adipocytes. However, it remains largely unknown how the body regulates leptin expression acutely and chronically in response to changes in physiological state such as adiposity. In the present study, we searched for diffusible regulators of leptin production from tissue extracts by taking advantage of a reporter cell line, Ob-Luc. Ob-Luc is a mouse adipocyte cell line that has a luciferase cassette knocked in at one allele of the leptin gene, which makes it possible to analyze leptin gene expression by high throughput luciferase assays. This search identified endothelin-1 (ET-1) as a leptin expression activator from rat brain extracts.

ET-1 is a 21-amino acid vasoactive peptide originally isolated from endothelial cell-conditioned medium (31). It induces constriction of vascular smooth muscle cells and is the most potent vasopressor known. In addition to its vascular effects, ET-1 exerts a wide variety of actions on many tissues. An elevated serum ET-1 concentration is associated with cardiovascular (32), renal (33), pulmonary (34), and hepatic diseases (35) as well as diabetes mellitus (36). ET-1 is produced primarily by the endothelium but also by a number of other cell types including neurons (37). In accordance with its multiple activities, ET-1 production has been shown to be regulated by many factors, such as growth factors and cytokines (38, 39), vasoactive substances (40), hypoxia (41), and mechanical forces (42). The endothelin family includes three isopeptides: ET-1, ET-2, and ET-3. ET-1 is as potent and efficacious as insulin in stimulating leptin production in adipocytes. In primary mouse white fat cells, we detected expression of both ET_A and ET_B by Northern blot and in situ hybridization analyses. We conclude that ET-1 stimulates leptin production via the ET_A receptor in cultured adipocytes.

The abbreviations used are: ob or Ob, obese; Luc, luciferase; ET, endothelin; ET_A and ET_B, endothelin A and B receptors; HPLC, high pressure liquid chromatography; DMEM, Dulbecco’s modified Eagle’s medium; PBS, fetal bovine serum; kb, kilobase; PBS, phosphate-buffered saline.

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Acetic acid and then eluted with 50% CH3CN, 0.1% trifluoroacetic acid. Peptides were eluted with a 100-min linear gradient of 15–45% CH3CN in 0.1% trifluoroacetic acid. Fractions were collected at 1-min intervals and assayed. The active fraction was pooled, diluted 4-fold with 0.1% trifluoroacetic acid, and loaded onto a C18 reverse-phase column, Vydac 219TP54 (4.6 mm x 250 mm), preequilibrated with 0.1% trifluoroacetic acid, and loaded onto a diphenyl reverse-phase column, Vydac 218TP510 (10 mm x 250 mm), preequilibrated with 0.1% trifluoroacetic acid. A 13–38% gradient of CH3CN in 0.1% trifluoroacetic acid was applied over 50 min at a flow rate of 1 ml/min. Fractions were collected at 1-min intervals and assayed. The active fractions were pooled, diluted 4-fold with 0.1% trifluoroacetic acid, and loaded onto a C18 reverse-phase column, Tosohaas TSK-CN 80Ts (4.6 mm x 250 mm), preequilibrated with 0.1% trifluoroacetic acid. A 23–43% gradient of CH3CN in 0.1% trifluoroacetic acid was applied over 60 min at a flow rate of 1 ml/min. Fractions were collected at 1-min intervals and assayed. The active fractions were pooled, diluted 4-fold with 0.1% trifluoroacetic acid, and loaded onto a C18 reverse-phase column, Vydac 218TP510 (10 mm x 250 mm), preequilibrated with 0.1% trifluoroacetic acid. A 13–38% gradient of CH3CN in 0.1% trifluoroacetic acid was applied over 50 min at a flow rate of 1 ml/min. Fractions were collected at 1-min intervals and assayed. The active fraction was subjected to a final round of HPLC using Amersham Pharmacia Biotech μRPC C2/C18 (2.1 mm x 100 mm) column, Vydac 218TP510 (10 mm x 250 mm), preequilibrated with 0.1% trifluoroacetic acid. The activity was eluted with a 45-min linear gradient of 20–50% CH3CN in 0.1% trifluoroacetic acid at a flow rate of 0.3 ml/min. The active fraction, which was collected manually, was subjected to electrospray mass spectrometry on a Micromass Quattro II spectrometer. The N-terminal amino acid sequence of the purified peptide was determined by an Applied Biosystems Model 494 protein sequencer.

**EXPERIMENTAL PROCEDURES**

**Purification of an Endogenous Stimulator of Leptin Production from Rat Brains**—Peptide extract was prepared as previously described (47). Briefly, about 270 g of frozen rat brains (150 pieces, Pel-Freeze) were homogenized in a 10× volume of homogenization solution (70% acetone, 1 M acetic acid, 20 mM HCl) and centrifuged at 20,000 × g for 30 min at 4 °C. The resulting supernatant was collected and extracted three times with an equal volume of diethyl ether. The aqueous phase was centrifuged again, and the supernatant was loaded onto six 10-g cartridges of Sep-Pak C18 (Waters), which were preequilibrated with 0.1% trifluoroacetic acid and then eluted with 50% CH3CN, 0.1% trifluoroacetic acid. The eluate was lyophilized and redissolved in 1 M acetic acid. The extract was loaded onto a C18 reverse-phase HPLC column, Vydac 218TP510 (10 mm x 250 mm), preequilibrated with 0.1% trifluoroacetic acid. Peptides were eluted with a 100-min linear gradient of 15–45% CH3CN in 0.1% trifluoroacetic acid at a flow rate of 4 ml/min. Fractions were collected at 1-min intervals and assayed. The active fraction was subjected to a final round of HPLC using Amersham Pharmacia Biotech μRPC C2/C18 (2.1 mm x 100 mm) column, Vydac 218TP510 (10 mm x 250 mm), preequilibrated with 0.1% trifluoroacetic acid. The activity was eluted with a 45-min linear gradient of 20–50% CH3CN in 0.1% trifluoroacetic acid at a flow rate of 0.3 ml/min. The active fraction, which was collected manually, was subjected to electrospray mass spectrometry on a Micromass Quattro II spectrometer. The N-terminal amino acid sequence of the purified peptide was determined by an Applied Biosystems Model 494 protein sequencer.

**Cell Culture**—The Ob-Luc preadipocyte cell line was generated to enable ex vivo cell-based transcription assays using adipocytes from transgenic animals having a “knock-in” luciferase reporter at one ob allele (48). The cell line was established from 14-day chimeric knock-in embryos, in which a luciferase reporter was inserted into the second exon of the leptin gene via standard gene targeting technique (48). Ob-Luc cells were grown in M3:20 media (InCell) and fed every 3 days. When confluent (day 0), cells were given fresh M3:20 media containing

![Graph A](Image)

**FIG. 1. Purification of ET-1 as an activator of leptin expression.** A, C18 reverse-phase HPLC (Vydac 218TP510, 10 mm x 250 mm) elution profile of the peptide extract. B, final purification of the active compound by an Amersham Pharmacia Biotech μRPC C2/C18 (2.1 mm x 100 mm) column. Elution was performed as described under “Experimental Procedures.” An arrow points to the active peak.

Here we report that ET-1 stimulates leptin expression in two different adipocyte cell lines, Ob-Luc and 3T3-L1. Furthermore, the stimulatory action of ET-1 primarily involves ETA signaling. In reporter assays, ET-1 acts as potently as insulin and positively interacts with insulin in stimulating leptin gene expression. We also detected expression of both ETA and ETB receptors in mouse white adipose tissues.

**FIG. 2. Endothelin increases leptin expression in Ob-Luc cells.** A, Northern blot analysis demonstrating leptin expression levels in Ob-Luc cells treated with PBS control, ET-1, or insulin. The mRNA was isolated from Ob-Luc cells treated with the indicated ligands for 4 h and hybridized with mouse leptin cDNA probe (upper panel). 1 μg of poly(A)-rich RNA was loaded, and equal amounts of loading were confirmed by stripping the membrane and rehybridizing with mouse β-actin probe (lower panel). B, dose-response curve of ET-1, ET-3, and insulin in increasing luciferase activity in Ob-Luc cells. The luciferase assay was performed with Ob-Luc cells incubated with ligands for 14 h. Values, which are expressed using light units as an indicator of luciferase activity, are the means of two experiments performed in triplicate.
50 μM 2-bromopalmitate (Aldrich). On day 3, the medium was switched to high glucose DMEM (Life Technologies, Inc.) containing 10% fetal bovine serum (FBS) and 10 mM dexamethasone. On day 2, medium was changed to DMEM containing 10% FBS, and cells were given fresh medium every 2 days until day 10. On day 12, cells were plated into a 96-well plate for luciferase assay or a 100-mm dish for RNA extraction.

3T3-L1 cells were grown in DMEM containing 10% FBS and fed every 3 days. One to two days after confluence (day 0), medium was switched to DMEM supplemented with 10% FBS, 10 μg/ml insulin, 0.25 mM 3-isobutyl-1-methylxanthine, and 1 mM dexamethasone. On day 2, medium was changed to DMEM containing 10% FBS, ET-3, and insulin in stimulating leptin secretion in 3T3-L1 cells. Fully differentiated 3T3-L1 cells were plated and incubated with ligands for 24 h, and culture media were taken for mouse leptin immunoassay. Data are expressed as immunoreactive leptin (pg) per ml of media. Values are the means of two experiments performed in duplicate.

50 μM 2-bromopalmitate (Aldrich). On day 3, the medium was switched to high glucose DMEM (Life Technologies, Inc.) containing 10% fetal bovine serum (FBS) and 10 μg/ml insulin. On day 5, cells were plated into a 96-well plate for luciferase assay or 100-mm dishes for RNA extraction.

3T3-L1 cells were grown in DMEM containing 10% FBS and fed every 3 days. One to two days after confluence (day 0), medium was switched to DMEM supplemented with 10% FBS, 10 μg/ml insulin, 0.25 mM 3-isobutyl-1-methylxanthine, and 1 mM dexamethasone. On day 2, medium was changed to DMEM containing 10% FBS and 10 μg/ml insulin. Beginning on day 4, medium was changed to DMEM containing only 10% FBS, and cells were given fresh medium every 2 days until day 12. On day 12, cells were plated into a 96-well plate for luciferase assay or a 100-mm dish for RNA extraction.

Luciferase Assay—Ob-Luc cells were plated in opaque 96-well plates (Corning). After 24 h, HPLC fractions or test compounds were added to wells, and cells were further incubated for 14 h. After incubation, the medium was removed, and 50 μl of assay buffer (30 mM tricine (pH 7.8), 8 mM MgCl₂, 0.2 mM EDTA, 100 mM 2-mercaptoethanol, 1 mM Triton X-100, 1.5 mM ATP, 0.5 mM coenzyme A, and 0.5 mM luciferin) were added to each well. After a 1-min incubation, luminescence was measured with a Torcon AML-34 luminometer. Sources of test compounds are as listed: insulin (Sigma); ET-1, ET-3, and insulin in stimulating leptin secretion in 3T3-L1 cells. Fully differentiated 3T3-L1 cells were plated and incubated with ligands for 24 h, and culture media were taken for mouse leptin immunoassay. Data are expressed as immunoreactive leptin (pg) per ml of media. Values are the means of two experiments performed in duplicate.

Leptin Immunoassay—Immuonassay of leptin in cell culture media was performed using a mouse leptin immunoassay kit (R & D Systems) as instructed by the manufacturer. 3T3-L1 cells were plated in a 96-well plate and incubated for 24 h to allow them to attach and recover. Cells were then fed fresh DMEM, 10% FBS containing the indicated compounds and incubated for 24 h, and media were collected and frozen at −80 °C for immunoassay.

Isolation of Mouse Primary Adipocytes—Isolation of mouse adipocytes was performed as described previously (49), with some modifications. Briefly, the epididymal fat pad was dissected from mice fed ad libitum. The fat pad was minced and digested with 5 mg/ml collagenase (Sigma) in Krebs-Ringer HEPES buffer (135 mM NaCl, 2.2 mM CaCl₂, 1.25 mM MgSO₄, 0.45 mM KH₂PO₄, 2.17 mM Na₂HPO₄, 10 mM HEPES, 5 mM d-glucose, 2% bovine serum albumin) at 37 °C for 2 h with gentle shaking every 5 min. Digested fat tissues were filtered through a 500-μm and then a 100-μm nylon mesh (Small Parts) and centrifuged at 2,000 × g for 10 min. The precipitated stromal-vascular cells and top lipid layer were removed, and fat cells were washed with Krebs-Ringer HEPES buffer three times. Packed fat cells were frozen at −80 °C for RNA isolation.

RNA Extraction and Northern Blot Analysis—Total RNA was extracted with the RNA STAT-60 kit (Tel-Test). Poly(A)⁺-rich RNA was prepared from total RNA with the Oligotex mRNA mini kit (Qiagen). For Northern blot analysis, RNA samples were electrophoresed in a 1% formaldehyde denaturing agarose gel, blotted onto a Hybond-N⁺ membrane (Amersham Pharmacia Biotech), and hybridized with specific probes using Quickhyb solution (Stratagene). Probes consisting of a 0.9-kb fragment of mouse ET₄ cDNA, a 0.35-kb fragment of mouse ET₅
cDNA, a 0.5-kb fragment of mouse leptin cDNA, and a 0.4-kb fragment of mouse β-actin cDNA were labeled with a Prime It II kit (Stratagene) in the presence of [32P]dCTP.

In Situ Hybridization—
Sectional in situ hybridization analysis was performed as described previously (50). Omental and epididymal white fat sections were taken from male C57BL/6J mice fasted for 24 h. A 0.35-kb fragment of mouse ETA cRNA, a 0.38-kb fragment of mouse ETB cRNA, and a 0.5-kb fragment of mouse leptin cRNA were used as probes. Riboprobes were generated with T7 RNA polymerase using a Maxiscript kit (Ambion) in the presence of both 35S-CTP and 35S-UTP.

RESULTS
Purification of ET-1 as an Activator of Leptin Production—
We screened tissue extracts for diffusible endogenous factors that increase expression of the leptin gene. Luciferase reporter assay was used to detect transcription activity in Ob-Luc adipocytes. Activity was detected in rat brain extract fractionated with a C18 reverse-phase column (Fig. 1A). We purified this activity through four additional steps of reverse-phase HPLC (see “Experimental Procedures”). The purified active fraction (Fig. 1B) was subjected to electrospray mass spectrometry and Edman microsequencing. The N-terminal sequence was CSCSSLMDKECVYF . . . , and the molecular mass was determined to be 2491.8 Da. These results revealed that the active substance was ET-1.

We confirmed the up-regulation of luciferase activity in response to synthetic ET-1 peptide in differentiated Ob-Luc cells. ET-1 increased the luciferase activity in a dose-dependent manner, as did ET-3 and insulin. The EC50 values for ET-1, ET-3, and insulin were 8.8 nM, 0.84 μM, and 55 nM, respectively (Fig. 2B). ET-1 failed to increase luciferase activity in a control HEK293 cell line in which a plasmid containing the same luciferase cassette was stably transfected. To further confirm that ET-1 can increase leptin expression, mRNA was extracted from Ob-Luc cells treated with vehicle, 100 nM ET-1, or 100 nM insulin and hybridized with a cDNA probe specific for leptin. ET-1 and insulin significantly increased leptin mRNA level in Ob-Luc cells (Fig. 2A).

ET-1 Stimulates Leptin Production in 3T3-L1 Cells—
To show that the stimulation of leptin expression by ET-1 is unique to the Ob-Luc cell line, we examined the effects of ET-1 on leptin expression in 3T3-L1 adipocytes by Northern blot analysis and leptin immunoassay. Differentiated 3T3-L1 cells were incubated with 10–100 nM ET-1 or 10 nM insulin for 4 h. Total RNA was extracted and hybridized with a cDNA probe specific for leptin. ET-1 and insulin significantly increased leptin mRNA level in 3T3-L1 cells (Fig. 3A). Furthermore, ET-1 increased leptin levels in the culture medium after a 24-h incubation. The EC50 values of ET-1, ET-3, and insulin were 4.1 nM, 8.4 μM, and 1.9 nM, respectively (Fig. 3B). In contrast to Ob-Luc cells, insulin was more potent than ET-1 in 3T3-L1 cells. This is probably caused by the presence of the Ob-Luc cell-specific ligand ET-1 peptide.
White Fat Cells Express Both ET\(_A\) and ET\(_B\) Receptors—We observed expression of the ET\(_A\) receptor in Ob-Luc cells and 3T3-L1 cells (Fig. 4). This is consistent with previous reports that ET\(_A\) is expressed in isolated rat adipocytes (53) and 3T3-L1 cells (54). Expression of ET\(_A\) and ET\(_B\) receptor mRNA on white fat cells from mice was examined by Northern blot analysis. RNA was extracted from adipocytes isolated from mouse epididymal fat (see “Experimental Procedures”) and hybridized with cDNA probes specific for mouse ET\(_A\) and ET\(_B\). We detected abundant expression of ET\(_A\), but not ET\(_B\), mRNA in both cell lines (Fig. 4A).

Next, we preincubated cells for 30 min with various concentrations of FR139317, an ET\(_A\)-selective antagonist, or BQ788, an ET\(_B\)-selective antagonist, and then stimulated the cells with 100 nM ET-1. FR139317 was significantly more potent than BQ788 in inhibiting ET-1-induced leptin expression. In Ob-Luc cells, the IC\(_{50}\) of FR139317 and BQ788 were 15 nM and 3.9 \(\mu\)M, respectively (Fig. 4B). In 3T3-L1 cells, the IC\(_{50}\) of FR139317 and BQ788 were 67 nM and 0.67 \(\mu\)M, respectively (Fig. 4C). These findings indicate that the stimulatory action of ET-1 involves ET\(_A\) signaling.

**ET-1 and Insulin Positively Interact with Each Other in Stimulating Leptin Production**—Insulin is a well known activator of leptin expression (25). Previous studies demonstrate that insulin enhances the ability of ET-1 to bind smooth muscle cell and stimulate cell proliferation (51), and ET-1 modulates insulin signaling through the phosphatidylinositol 3-kinase pathway (52). To investigate whether ET-1 and insulin cross-talk with each other in stimulating leptin expression in adipocytes, Ob-Luc cells and 3T3-L1 cells were stimulated with various concentrations of ET-1 in the presence of insulin. In Ob-Luc cells, both 3 and 10 nM insulin increased the efficacy of ET-1 (Fig. 5B). Insulin increased the maximum effect of ET-1 but did not change the EC\(_{50}\) value. This effect of insulin is not merely additive. Low concentrations (1 and 3 nM) of ET-1 also increased the efficacy of insulin without changing the EC\(_{50}\) value of the insulin dose-response curve (Fig. 5C). In 3T3-L1 cells, 10 nM insulin increased the potency of ET-1, i.e. decreased the EC\(_{50}\) value (0.7 nM compared with 4.3 nM) (Fig. 6B). Ob-Luc cells stimulated with 100 nM insulin and 100 nM ET-1 together had a higher leptin expression level than cells stimulated solely with 100 nM insulin or 100 nM ET-1 (Fig. 5A). Similarly, 3T3-L1 cells expressed more leptin mRNA when stimulated with 10 nM insulin and 100 nM ET-1 than when stimulated by either 10 nM insulin or 100 nM ET-1 alone (Fig. 6A).

**The Stimulatory Action of ET-1 Involves ET\(_A\) Signaling**—ET-1 was significantly more potent in stimulating leptin expression than ET-3 in both Ob-Luc and 3T3-L1 cells. ET\(_A\) is selective for ET-1 over ET-3, whereas ET\(_B\) has similar affinities for ET-1 and ET-3. Therefore, we felt that the stimulatory action of ET-1 probably involves ET\(_A\) signaling. To confirm that ET-1 stimulates leptin expression via the ET\(_A\) receptor, we observed the expression of ET\(_A\) and ET\(_B\) in adipocyte cell lines. RNA extracted from differentiated Ob-Luc cells and 3T3-L1 cells was hybridized with cDNA probes specific for mouse ET\(_A\) and ET\(_B\). We detected abundant expression of ET\(_A\), but not ET\(_B\), mRNA in both cell lines (Fig. 4A).
synthetic ET-1 stimulates leptin production in two distinct adipocyte cell lines, Ob-Luc and 3T3-L1 cells. ET-1 acts via ETA and is as potent as insulin in these cells. Furthermore, both ETA and ETB receptors are expressed in white adipose tissues in mice.

When undertaking this study, we did not suspect that ET-1 stimulates leptin expression. It is well established that leptin is a hormone involved in energy homeostasis, whereas ET-1 is a potent vasoconstrictor. To our knowledge, this is the first report directly linking leptin and endothelin. Recently it was revealed that leptin receptor (OB-Rb) is expressed in endothelial cells and that leptin promotes angiogenesis (16). Leptin also has profound sympathetic, vascular, and renal actions, and leptin-overexpressing transgenic mice are lean but hypertensive due to hyperleptinemia (55). In leptin-transgenic obese KKAv mice, caloric restriction reduces only body weight but not blood pressure, whereas it improves both obesity and hypertension in non-transgenic KKAv mice (56). On the other hand, ET-1 is known to act on adipocytes, affecting adipogenesis (57, 58) and glucose metabolism (59, 60). Interestingly, leptin and ET-1 production are both activated by insulin (25, 61, 62) and inhibited by peroxisome proliferator-activated receptor-γ agonists (30, 63), suggesting an intimate relationship among these factors.

ET-1 stimulates leptin expression in Ob-Luc and 3T3-L1 cells through ETA in a dose-dependent manner. The EC50 of this ET-1 effect was comparable with the EC50 of insulin to stimulate leptin expression as well as with the EC50 of ET-1 to induce vasconstriction. This is also consistent with previous reports that adipocytes express ETA (53, 54). However, we detected expression of both ETA and ETB in native white mouse fat cells by both Northern blot and in situ hybridization analyses. Despite the involvement of ETA in the stimulation of leptin production in adipose cell lines, ETB is expressed at a higher level than ETA in native fat cells. This may indicate a different stage of differentiation of ETA and ETB receptors at different stages of adipogenesis. Further studies are needed to elucidate expression patterns of endothelin receptors in adipocytes in various states and to clarify how endothelin affects leptin production through these receptors.

We also showed that ET-1 positively interacts with insulin in stimulating insulin production. Physiological concentrations of ET-1 seem to modulate the amplitude of the well known stimulatory effect of insulin on leptin production in adipocytes. Insulin and ET-1 share common signal transduction pathways such as phosphatidylinositol 3-kinase (52), and both peptides can affect expression levels of insulin receptor substrate-1 and insulin receptor-β (64). Insulin also augments ET-1 activity on vascular smooth muscle cells (51). The mechanism and physiological significance of this interaction will be the subject of further studies.

It is well recognized that body energy homeostasis impacts on the cardiovascular system. Metabolic disorders such as obesity and diabetes mellitus are high risk factors associated with cardiovascular disease and hypertension. However, little is known about how the vascular system affects energy homeostasis. Our findings suggest that vascular factors such as ET-1 may regulate the metabolic system through leptin. This line of investigation may help to elucidate the physiological and pathological roles that ET-1 and leptin play in the cross-talk between cardiovascular and metabolic systems.

Acknowledgments—We thank Steve McKnight for discussion and Cheryl Gariety, David Clouthier, and Jon Willie for critically reading the manuscript.

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J. Biol. Chem. 2001, 276:28471-28477.
doi: 10.1074/jbc.M103478200 originally published online May 18, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M103478200

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