Leptin reduces plasma ANP level via nitric oxide-dependent mechanism

Kuichang Yuan,¹* Jiahua Yu,²* Amin Shah,¹ Shan Gao,¹ Sun Young Kim,¹ Sung Zoo Kim,¹ Byung-Hyun Park,² and Suhn Hee Kim¹

Departments of ¹Physiology and ²Biochemistry, Diabetic Research Center and Medical School, Chonbuk National University, Jeonju, Jeonbuk, Republic of Korea

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Yuan K, Yu J, Shah A, Gao S, Kim SY, Kim SZ, Park BH, Kim SH. Leptin reduces plasma ANP level via nitric oxide-dependent mechanism. Am J Physiol Regul Integr Comp Physiol 298: R1007–R1016, 2010. First published January 13, 2010; doi:10.1152/ajpregu.00598.2009.—Leptin is a circulating adipocyte-derived hormone that influences blood pressure (BP) and metabolism. This study was designed to define the possible role of leptin in regulation of the atrial natriuretic peptide (ANP) system using acute and chronic experiments. Intravenous infusion of rat leptin (250 μg/kg injection plus 2 μg·kg⁻¹·min⁻¹ for 20 min) into Sprague-Dawley rats increased BP by 25 mmHg and decreased plasma level of ANP from 80.3 ± 3.45 to 51.8 ± 3.3 pg/ml. Reserpination attenuated the rise in BP, but not the reduction of plasma ANP during leptin infusion. N⁶-nitro-L-arginine methyl ester prevented the effects of leptin on the reduction of ANP level. In hyperleptinemic rats that received adenosine containing rat leptin cDNA (AdCMV-leptin), BP increased during first 2 days and then recovered to control value. Plasma concentration of ANP and expression of ANP mRNA, but not of atrial ANP, in hyperleptinemic rats were lower than in the control groups on the first and second week after administration of AdCMV-leptin. These effects were not observed by the pretreatment with N⁶-nitro-L-arginine methyl ester. No differences in renal function and ANP receptor density in the kidney were found between hyperleptinemic and control rats. These data suggest that leptin inhibits ANP secretion indirectly through nitric oxide without changing basal or isoproterenol-induced ANP secretion.

sympathetic nervous system; atrial natriuretic peptide; adenosine 3',5'-cyclic monophosphate; hypertension; receptor

LEPTIN IS A CIRCULATING adipocyte-derived hormone that regulates energy balance through binding to leptin receptors (Ob-R) at the hypothalamus (37). Leptin decreases appetite and increases body temperature and energy consumption, thereby decreasing adipose tissue mass and body weight (BW) (11, 28, 32). The Ob-R is a member of the extended class I cytokine receptor family, with at least six splice variants, Ob-R (a–f) (27), and is distributed in various tissues, such as lung, kidney, heart, vascular endothelium, and brain (23, 31). Wide distributions of the Ob-R and leptin mRNA suggest a diversity of its functions. In addition to the well-known metabolic functions of leptin, it also regulates autonomic, cardiovascular, endocrine, and renal functions. However, these functions are still controversial and are under investigation (22). Recent studies have focused on the potential effects of leptin on blood pressure (BP) and renal function through activation of the sympathetic nervous system (SNS). Dose-dependent increases in sympathetic discharge from the kidney and brown adipose tissue by murine leptin have been reported (15). Acute intravenous infusion of leptin showed no effect on BP (15, 16), whereas chronic infusion of murine leptin caused an increase in BP and heart rate (HR), as well as renal vascular resistance (6). Hypertension was developed in transgenic mice overexpressing leptin (2). However, the relationship between leptin-mediated hypertension and activation of SNS remains to be understood.

Atrial natriuretic peptide (ANP) is an important antagonist of the renin-angiotensin system in regulation of body fluid and BP. ANP is released mainly from atrial myocytes into the bloodstream in response to stretch (10, 13, 22), causing diuresis, natriuresis, and vasodilation (7). Impairment of ANP release or change in its biological receptors causes hypertension or cardiac hypertrophy (20, 25). A recent study has suggested an influence of leptin on ANP secretion (20). Thus, ob/ob leptin-deficient mice with transverse aortic constriction enhanced cardiac hypertrophy and blunted an increased ANP gene in the ventricle (20). Either intraperitoneal injection of leptin in ob/ob mice or exposure of leptin in rat cardiac cell lines activated the ANP promoter via the nuclear factor activated T-cell signaling pathway (20). In contrast to the antihypertrophic role of leptin, a pro-hypertrophic role of leptin has also been reported (34). This study was designed to evaluate changes in the ANP system and in BP with acute or chronic exposure of rats to leptin.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, weighing 200–250 g, were obtained from the Orientbio (Seoungnam, Republic of Korea). Animals were housed in a temperature-controlled room with a 12:12-h light-dark cycle. Animals were provided free access to standard laboratory chow (SL79 Purina rat and mouse 18% chow, Charles River Laboratories, Wilmington, MA) and water. All of the experimental protocols conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85–23, revised 1996) and were approved by our Institution’s Ethical Review Board.

Acute effects of leptin on hemodynamics and plasma ANP levels. Under anesthesia using xylazine and ketamine (1:9, 2 ml/kg), a polyethylene tube was cannulated into the carotid artery for measurement of BP and HR and into the external jugular vein for the infusion of leptin. Following a 30-min period for stabilization, 800 μl of blood were collected in an Eppendorf tube. Rat leptin was injected at a dose of 250 μg/kg, followed by infusion of rat leptin at 2 μg·kg⁻¹·min⁻¹ for 20 min using a peristaltic pump at a rate of 60 μl/min (24). Physiological saline, instead of leptin, was infused into the control group. Blood was collected after stopping the infusion. BP and HR were recorded using a Power Lab (ML-820, ADInstruments) via a pressure transducer (Statham P23Db, Oxnard, CA). After centrifugation of blood samples at 10,000 g at 4°C, plasma was collected and kept at –20°C until use.

To modulate the effects of leptin on BP and plasma ANP level, reserpine (3 mg·kg⁻¹·day⁻¹) (1) was injected intraperitonely for 3 days. Under anesthesia using xylazine and ketamine, rat leptin was then

* K. Yuan and I. Yu contributed equally to this work.

Address for reprint requests and other correspondence: S. H. Kim, San 2-20, Keum-am-dong, Dept. of Physiology, Chonbuk National Univ. Medical School, Jeonju, Jeonbuk, 561-756, Korea (e-mail: shkim@chonbuk.ac.kr).

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injected at a dose of 250 µg/kg, followed by infusion of rat leptin at 2 µg·kg⁻¹·min⁻¹ for 20 min (24). The BP and HR were measured, and blood was collected using the protocol described above.

To modulate the effects of leptin on BP and plasma ANP level, rats were pretreated with the addition of Nω-nitro-L-arginine methyl ester (l-NAME), an inhibitor of nitric oxide (NO) synthase (25 mg/rat) (21) to their drinking water for 7 days before infusion of leptin.

For constant maintenance of plasma ANP level, rat leptin was injected at a dose of 250 µg/kg, followed by infusion of rat leptin at 2 µg·kg⁻¹·min⁻¹ and ANP at 5 ng·kg⁻¹·min⁻¹ for 20 min using a peristaltic pump at a rate of 60 µl/min.

**Chronic effects of leptin on food intake, renal function, and ANP level.** Rats were placed in metabolic cages (Tecniplast), with free access to food and water. After adaptation to metabolic cages over a period of 3 days, urine was collected every 24 h for 3 days at room temperature, and rats then received recombinant adenosine containing either leptin cDNA (AdCMV-leptin) or, as a control, β-galactosidase cDNA (AdCMV-β-gal), prepared as described (28). Under anesthesia with xylazine and ketamine, 3 × 10¹² plaque-forming units of adenovirus were administered via a lingual vein. Pair-fed rats, as a control, were supplied as much as hyperleptinemic rats fed and drank. Food and water intake and urine volume were monitored for 1 wk. Water balance was obtained by subtraction of urine volume from water intake. Urinary sodium and potassium concentrations were determined using a flame photometer (ELEKTA 636I; Eppendorf, Hamburg, Germany). After 1 wk, rats were killed by decapitation, and blood was collected into vials containing 50 µl of 0.1 M ethylenediamine tetraacetic acid, centrifuged at 4°C at 10,000 g for 15 min, and stored at −20°C until assay. Atrial tissues for measurement of ANP mRNA and ANP content were kept at −70°C until assay.

**Chronic effects of leptin on hemodynamics.** Under anesthesia with xylazine and ketamine, a telemetric pressure-reading transmitter (PAC-40, Data Sciences International, St. Paul, MN) was implanted into the abdominal aorta, according to the manufacturer’s protocol, and both the abdominal muscular layer and the skin layer were then closed (35). To regain their preoperative BW, rats were housed individually at room temperature and allowed to recover for 5 days. After recovery, BP and HR in conscious, unrestrained rats were measured continuously for 3 days (PhysioTel Telemetry System, Data Sciences International), and 3 × 10¹² plaque-forming units of adenovirus were then administered into a lingual vein. Pair-fed rats, as a control, were supplied as much as hyperleptinemic rats fed and drank. BP and HR were measured continuously for 1 wk, and data were later analyzed offline.

**Chronic effects of leptin on ANP level in l-NAME-treated rats.** To modulate chronic effects of leptin on ANP level, rats were pretreated with the addition of l-NAME, an inhibitor of NO synthase (25 mg/rat) (21), to their drinking water for 7 days before the experiment, and 3 × 10¹² plaque-forming units of adenovirus were then administered into a lingual vein under xylazine and ketamine anesthesia. Pair-fed rats, as a control, were supplied as much as hyperleptinemic rats fed and drank. Rats received continuously l-NAME for 7 days. After 1 wk, rats were killed by decapitation, and blood was collected into vials containing 50 µl of 0.1 M ethylenediamine tetraacetic acid, centrifuged at 4°C at 10,000 g for 15 min, and stored at −20°C until assay. Atrial tissues for measurement of ANP mRNA and ANP content were kept at −70°C until assay.

**Chronic effects of leptin on basal and stimulated ANP secretion by isoproterenol from isolated atria of hyperleptinemic rats.** Isolated, perfused, beating atria were prepared using a previously described method (14). One week after virus infection, rats were killed by decapitation, and the left atrium was dissected from the heart. A cannula was inserted into the atrium and secured by ligatures. The cannulated atrium was transferred into an organ chamber, perfused with oxygenated HEPES buffer solution (10 mM HEPES, 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 25 mM NaHCO₃, 10 mM glucose, and 0.1% bovine serum albumin, pH 7.4) at 36.5°C, and paced at 1.3 Hz (duration 0.3 ms, voltage 40 V). Intra-atrial pressure was recorded using a Power Lab (ML-820, ADInstruments) via a pressure transducer (Statham P23Db), and pulse pressure was determined from the difference between systolic and diastolic pressure. Pericardial buffer solution, which contained [³H]inulin (Amersham Biosciences, Aylesbury, UK) for measurement of translocation of extracellular fluid (ECF), was oxygenated by placement of silicone tube coils inside the organ chamber. After stabilization for 100 min, atrial perfusate was collected at 2-min intervals at 4°C for 10 min, isoproterenol (3 nM) (36) was introduced into the atrial lumen, and perfusates were collected. cAMP concentrations in perfusates were measured during the three control periods and the final three experimental periods.

To evaluate direct effects of leptin on the ANP secretion, rat leptin (20, 100, and 500 nM) was perfused into isolated, beating atria from control rats after five collection periods, and perfusate was then collected continuously, as described above.

**Measurement of ECF translocation.** Radioactivity of [³H]inulin in atrial perfusate was measured using a liquid scintillation counter (Tris-Carb 23-TR, A Packard Bioscience, Downers Grove, IL). The amount of ECF translocated through the atrial wall was calculated by dividing total radioactivity in perfusate by radioactivity in the pericardial lumen. Resulting values were expressed in microliters per minute per gram atrial tissue (8). Since ANP is secreted into the atrial lumen with the translocation of ECF, we calculated interstitial ANP concentration using the following equation:

\[
\text{ANP concentration (µM)} = \frac{\text{ANP (pg·min}^{-1}·g^{-1})}{\text{ECF translocation (µl·min}^{-1}·g^{-1})} \times 3,060
\]

Because the ANP secretion was the processed ANP, the denominator, 3,060, refers to the molecular mass for ANP (1,285 Da).

**Measurement of ANP, CAMP, leptin, renin, and catecholamine concentrations.** Atria were removed, weighed, and placed in 2 ml of 0.1 M acetic acid. After boiling for 10 min, each atrium was homogenized using a Polytron homogenizer (PT-1200, Kinematica) and then centrifuged at 4°C at 10,000 g for 15 min (10). Plasma ANP was extracted using a Sep-Pak C₁₈ cartridge and then dried using a Speed-Vac concentrator (Savant, Farmingdale, NY) (9). Concentrations of ANP in tissue, plasma, and perfusate were measured using a specific RIA, as described previously (9).

For measurement of CAMP in perfusate, 100 µl of perfusate were treated with trichloroacetic acid (300 µl) to a final concentration of 6% for 15 min at room temperature and centrifuged at 4°C. The supernatant (100 µl) was transferred to a polypropylene tube, extracted three times with water-saturated ether, and then dried using a Speed-Vac concentrator (Savant). The dried samples were resuspended with sodium acetate buffer, and production of cAMP was measured using a specific RIA, as previously described (12).

Concentration of plasma leptin was also measured using a RIA with a commercial kit (Linco Research, Billerica, MA).

Plasma renin concentration (PRC) was measured using RIA (9). Plasma catecholamine was measured using a radioenzymatic method.

In vitro autoradiographic binding of [³H]-l-ANP. For in vitro receptor autoradiography, kidneys were rapidly removed and snap frozen in liquid nitrogen. Sections (20 µm) of kidneys were cut in a cryostat in a transverse plane, thaw-mounted on gelatin-chrom-alum-coated slides, and then dried in desiccators overnight. Binding of [³H]-labeled ANP to transverse kidney sections was evaluated under conditions previously described (17). Briefly, sections were washed with 150 mM NaCl-0.5% acetic acid (pH 5.0) at room temperature for 10 min for removal of endogenous ANP and then preincubated with 30 mM phosphate buffer (pH 7.2) containing 120 mM NaCl and 1 mM phenolthalenine at room temperature for 8 min. Sections were then incubated with 250 pM of [³H]-l-ANP in fresh preincubation buffer containing 40 µg/ml bacitracin, 100 µg/ml phenylmethylsulfonyl fluoride, 10 µg/ml leupeptin, and 0.5% bovine serum albumin at room temperature for 60 min. For measurement
of binding constants, competitive inhibition of $^{125}$I-ANP binding of kidney was determined in consecutive sections by coincubation with various concentrations of unlabeled ANP [a ligand for natriuretic peptide receptor A (NPR-A)] or C-ANP (a ligand for NPR-C) and 250 pM of $^{125}$I-ANP. After incubation, sections were rinsed and washed with fresh preincubation buffer for 5 min at 4°C. They were subsequently rinsed three times in cold, distilled water at 4°C and quickly dried under a stream of cold air. Autoradiographic images were generated by exposure of slides to Hyperfilm-$^3$H (Amersham International) in X-ray cassettes at room temperature, and autoradiograms were then developed and fixed. Using the PRISM image program (version 3.6–1, Improve Vision, Coventry, UK), regional binding of $^{125}$I-ANP in the kidney was analyzed for a mean gray-scale value.

Real-time RT-PCR for ANP and $\alpha_{1A}$- or $\beta_1$-adrenergic receptor mRNA levels. Total RNA was extracted from rat atria tissue using TRIZol reagent (Invitrogen, Carlsbad, CA). Reverse transcription was performed using Superscript II and 18-mers oligo(dT) (Invitrogen) (35). Specific primers were designed using primer-express software, and their primer sequences were as follows: rat $\alpha_{1A}$-receptor (accession NM_017191.2), 5'-TGGCAGGGTGTTC TGCAATA-3' (forward), and 5'-GACGGTGCGCATGAT-3' (reverse); rat $\beta_1$-receptor (accession NM_012701.1), 5'-CCTCGTCCGTCGTCTCCTT-3' (forward), and 5'-AACACCCGGAGGTACACGAA-3' (reverse); rat actin (accession NM_031144.2), 5'-ACCAGTTCGCCATGGATGAC-3' (forward), and 5'-TGCCGGAGCCGTTC-3' (reverse). The real-time RT-PCR reaction was contained in a final volume of 10 μl that included 10 ng of reverse transcribed total RNA, 200 nM of forward and reverse primers, and 2 μl PCR master mix. The RT-PCR reaction was carried out in 384-well plates using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster, CA). All reactions were performed in triplicate.

Statistical analysis. Results are presented as means ± SE. Statistical differences were assessed using analysis of variance followed by the Bonferroni multiple-comparison test or the Duncan multiple-range test. Student’s t-test was also used. Statistical significance was set at $P < 0.05$.

RESULTS

Acute effects of leptin on BP, HR, and ANP levels. Figure 1 shows the acute effect of leptin on BP, HR, and plasma ANP level in control, reserpinized, and L-NAME-treated rats. Rat leptin was injected at a single dose of 250 μg/kg and then infused at a maintenance dose of 2 μg·kg$^{-1}$·min$^{-1}$ for 20 min.

Fig. 1. Effects of leptin on systolic blood pressure (BP; a), heart rate (HR; b), and plasma levels of atrial natriuretic peptide (PANP; c) in control (A), reserpine-treated (B), Nω-nitro-L-arginine methyl ester (L-NAME)-treated, (C) and atrial natriuretic peptide (ANP)-coinfused rats (D). Rats received either reserpine (3 mg·kg$^{-1}$·day$^{-1}$) or vehicle intraperitoneally for 3 days. Rats received L-NAME (25 mg/day) added to their drinking water for 7 days before the experiment. After anesthesia, rat leptin was injected at 250 μg/kg, and 2 μg·kg$^{-1}$·min$^{-1}$ was then infused for 20 min at a rate of 0.6 ml/10 min. ANP in the other group was coinfused with leptin for 20 min after leptin injection. Blood was collected before and after leptin infusion. Hatched bars indicate the leptin infusion periods, and shaded hatched bars indicate the leptin and ANP infusion periods. Δ, Change. Values are means ± SE. *$P < 0.05$ and **$P < 0.01$ vs. vehicle or control group; +$P < 0.05$, ++$P < 0.01$ vs. control value.
Plasma leptin level in control rats was 93.45 ± 8.27 ng/ml \((n = 5, P < 0.001)\) compared to 80.3 ± 3.5 pg/ml \((n = 5, P < 0.001)\) in the vehicle-infused group. Plasma concentration of ANP decreased from 80.3 ± 3.5 to 51.8 ± 3.3 pg/ml \((n = 5, P = 0.001)\) in the leptin-infused group and from 73.6 ± 8.2 to 64.5 ± 8.9 pg/ml \((n = 5, P = 0.05)\) in the vehicle-infused group. Pretreatment with reserpine blocked the leptin-induced increase of BP (Fig. 1 Ba), but not the leptin-induced decrease in plasma ANP level (Fig. 1 Bc).

To determine whether BP increased by leptin is due to low plasma ANP level, we attempted to maintain a constant plasma ANP level during leptin infusion. Different doses of ANP (2, 5, 10, or 20 ng·kg\(^{-1} \cdot \text{min}^{-1}\)) were infused for 20 min, and blood was collected before and after ANP infusion for measurement of plasma ANP level. We selected ANP dose, 5 ng·kg\(^{-1} \cdot \text{min}^{-1}\), which raised plasma ANP by ~50 pg/ml. Leptin was injected at a dose of 250 μg/kg, followed by infusion of leptin at 2 μg·kg\(^{-1} \cdot \text{min}^{-1}\) and ANP at 5 ng·kg\(^{-1} \cdot \text{min}^{-1}\) for 20 min. Leptin did not cause a significant increase in BP (Fig. 1 Da) during coinfusion with ANP.

**Chronic effects of leptin on BW, food intake, and renal function.** BW in hyperleptinemic rats decreased from 249 ± 1.3 to 234.0 ± 7.5 g \((n = 5, P = 0.001)\) during the first week, whereas BW in AdCMV-β-gal rats increased from 250.0 ± 1.5 to 284.3 ± 4.9 g \((n = 5, P = 0.001)\) (Fig. 2A). Food intake...
(g/100 g BW) in hyperleptinemic rats decreased compared with that in AdCMV-β-gal rats (Fig. 2B). Urine volume (ml/100 g BW) was similar among the three groups (Fig. 2C). Water balance (Fig. 2D) and urinary excreted amount of sodium and potassium (Fig. 2, E and F) in hyperleptinemic rats were lower than those in AdCMV-β-gal rats, but were not different from those of pair-fed rats.

**Chronic effects of leptin on hemodynamics and ANP system.** Plasma leptin level in AdCMV-leptin rats was 73.2 ± 4.2 ng/ml (n = 7, P < 0.01) during the first week, which was higher than that in pair-fed rats (1.5 ± 0.5 ng/ml, n = 5) and AdCMV-β-gal rats (2.4 ± 0.7 ng/ml, n = 5). BP and HR were continuously monitored before and after virus injection. Figure 3, A and B, shows representative mean values of SBP and HR obtained from rats injected with AdCMV-leptin (n = 5) and AdCMV-β-gal (n = 3). SBP in AdCMV-β-gal rats showed a tendency to decrease with time after virus injection, whereas SBP in AdCMV-leptin rats tended to increase during the first 2 days and then maintained the basal level. HR in both AdCMV-β-gal and AdCMV-β-gal rats maintained the basal level of HR (Fig. 3B). In hyperleptinemic rats, SBP increased from 99.4 ± 4.2 to 108.3 ± 5.6 mmHg (P < 0.01) and 105.1 ± 6.0 mmHg (n = 9, P < 0.05) during the first 2 days, respectively, and then returned to control level (99.5 ± 4.7 mmHg) during the 3rd day after virus injection. SBP in AdCMV-β-gal rats decreased from 104.8 ± 7.8 to 95.7 ± 9.9 mmHg (n = 6) during the 7th day after virus injection, and SBP in pair-fed rats showed no significant change (103.3 ± 4.4 to 103.3 ± 2.5 mmHg, n = 4) during the 7th day after food restriction. No significant difference in HR was found among the three groups.

During the first week after virus injection, plasma concentration of ANP (Fig. 3C) and expression of atrial ANP mRNA (Fig. 3E) in hyperleptinemic rats (n = 10) were significantly decreased compared with those in pair-fed (n = 10) and AdCMV-β-gal rats (n = 9). However, ANP content in atrial tissue was not altered (Fig. 3D). PRC in hyperleptinemic rats was higher than in pair-fed and AdCMV-β-gal rats (13.24 ± 1.40 vs. 6.45 ± 0.76 and 6.37 ± 0.85 ng angiotensin I·ml⁻¹·h⁻¹, P < 0.01). During the second week after virus injection, in addition to decreased plasma ANP level (42.9 ± 4.8 vs. 80.2 ± 6.8 and 86.5 ± 3.4 pg/ml, n = 6 for all groups, P < 0.01) and ANP mRNA, atrial tissue content of ANP was also decreased (30.3 ± 2.7 vs. 47.2 ± 1.7 and 47.5 ± 4.2 ng/mg tissue, n = 6 for all groups, P < 0.01). Pretreatment with L-NAME prevented the decreases in plasma ANP level (Fig. 3F) and atrial ANP mRNA (Fig. 3H) in hyperleptinemic rats (n = 6 for all groups).

**Changes in ANP binding sites in hyperleptinemic rat kidney.** To determine whether ANP binding sites in hyperleptinemic rats are changed, ANP binding sites were evaluated using 125I-ANP in rat kidney during the first week after virus injection. Binding sites...
for $^{125}$I-ANP were distributed in the cortex, inner medulla, and intrarenal arteries (Fig. 4A, left) and completely displaced in the presence of 1 μM unlabeled ANP (Fig. 4A, right) in hyperleptinemic rats. The ANP binding density was much higher in the cortex (Fig. 4B, left) than in the medulla (Fig. 4B, right). However, no difference in ANP binding density was found among the three groups (Fig. 4B). Figure 4C shows a comparison of competitive inhibition curves of specific ANP binding to glomeruli by unlabeled ANP (Fig. 4C, left) or C-ANP (Fig. 4C, right). No significant difference in the binding affinity was also found among three groups ($n = 5$ for all groups).

Changes in basal and stimulated ANP secretion by isoproterenol from isolated atria of hyperleptinemic rats. Activation of SNS by leptin, followed by an increased BP, has been reported (12, 19). Therefore, it is possible that ARs may be downregulated, or their signal pathway may be altered in atria of hyperleptinemic rat. To examine whether hyperleptinemic condition alters atrial response to an adrenergic hormone, an agonist of β-AR, isoproterenol (3 nM), was perfused into isolated, beating atria from hyperleptinemic ($n = 6$), pair-fed ($n = 5$), and AdCMV-β-gal rats ($n = 5$), including control rats ($n = 5$). As shown in Fig. 4A, basal atrial contractility, ECF translocation, and ANP secretion were not different among the four groups. In control rat atria, isoproterenol rapidly increased atrial contractility with an increased ECF translocation and then recovered to the control level at the end of the experiments (Fig. 5, Aa and Ab). ANP secretion and interstitial ANP concentration abruptly decreased, reached peak value at 20 min.

![Image](image-url)
after the start of isoproterenol, and recovered to the control levels (Fig. 5, Ac and Ad). Figure 5B shows percent changes in atrial parameters between the five control values and the three peak experimental values (fraction 8–10 for pulse pressure and ECF translocation, fraction 14–16 for ANP secretion and concentration) exposed to isoproterenol from Fig. 5A. Isoproterenol-induced increments of atrial contractility (Fig. 5Ba) and ECF translocation (Fig. 5Bb) in hyperleptinemic rat atria were not different from those in pair-fed, AdCMV-β-gal, and control rats. Isoproterenol-induced decrease in ANP secretion (Fig. 5Bc and Bd) and the increase in cAMP level in perfusate (Fig. 5Be) were similar among the four groups. In addition, no significant differences were observed in the levels of plasma catecholamine and atrial α1A- and β1-AR mRNA between hyperleptinemic and pair-fed rats (n = 4 for all groups, Fig. 6).

Fig. 5. A: effects of isoproterenol (ISP) on pulse pressure (a), extracellular fluid (ECF) translocation (transloc) (b), ANP secretion (c), and concentration (conc) (d) in terms of time in isolated, perfused atria from rats that received virus and control atria. After stabilization, atrial perfusate was collected at 2-min intervals at 4°C for 10 min. ISP (3 nM) was then introduced into the atrial lumen, and perfusates were collected continuously. B: comparison of atrial response to ISP in four groups. a: pulse pressure; b: ECF translocation; c: ANP secretion; d: ANP concentration; e: cAMP amount. Values were expressed as relative percent changes in atrial parameters between the five control values and the three peak experimental values (fraction 8–10 for pulse pressure and ECF translocation, fraction 14–16 for ANP secretion and concentration) exposed to ISP. Values are means ± SE. Arrows indicate the start of perfusion with ISP. Time-Cont, control rat atria perfused with HEPES buffer only; CONT-ISP, control rat atria perfused with ISP; Leptin-ISP, hyperleptinemic rat atria perfused with ISP.
DISCUSSION

In this study, we have evaluated roles of leptin in regulation of ANP synthesis and secretion and the ANP receptor expression using in vivo, ex vivo, and hyperleptinemic rat models. Results from in vivo study revealed that overexpression of leptin, as well as infusion of leptin, causes a decrease in plasma ANP level and an increase in BP. No significant changes in renal function, secretion rate of ANP, and density of the renal ANP receptor were found in hyperleptinemic rats. The effects of leptin infusion on ANP secretion and BP were inhibited by pretreatment with L-NAME, whereas perfusion of leptin into isolated atria had no effect on ANP secretion. Moreover, pretreatment with L-NAME inhibited the decreases in the levels of plasma ANP and atrial ANP mRNA in hyperleptinemic rats, but not in pair-fed and AdCMV-β-gal rats. Interestingly, ANP content was not changed under the experimental conditions used in this study. These observations suggest that the leptin-NO system modulates the ANP expression, but not the ANP secretion, thereby changing the plasma level of ANP.

It has been well demonstrated that circulating leptin crosses the blood-brain barrier and acts on the hypothalamus in regulation of energy balance via stimulation of sympathetic nerve activation (15). In the present study, plasma leptin level in AdCMV-leptin rats was ~70 ng/ml. Decreased food intake and BW in AdCMV-leptin rats is an indication that circulating leptin secreted from AdCMV-leptin may act properly in our system. In this study, we evaluated the question of whether leptin functions in regulation of ANP synthesis, secretion, and receptor. Our present study showed that acute injection of leptin reduces plasma ANP level, and leptin overexpression for 7 days reduces the levels of plasma ANP and atrial ANP mRNA expression without changing atrial ANP content. Stimulation of systemic NO release in rats (4, 5) and in endothelial cells and blood vessels during acute infusion of leptin has been reported (29, 33). NO is known to inhibit ANP secretion (13, 26). In our study, the pretreatment with L-NAME blocked the effects of leptin on the levels of plasma ANP and atrial ANP mRNA expression. Taken together, we suggest that leptin-NO signaling downregulates expression of ANP mRNA and, therefore, results in reduction of plasma ANP.

Leptin-induced activation of SNS has been reported (24). We recently reported an opposite regulation of ANP secretion by atrial α1A- and β1-agonists (36). Therefore, we tested a possible involvement of β-AR in reducing ANP secretion in hyperleptinemic rats. Our results negated this possibility. Perfusion of isoproterenol, a β-agonist, into atria isolated from hyperleptinemic rats resulted in suppression of ANP secretion that was similar to that of atria from pair-fed rats. Basal ANP secretion was also similar in two groups. No significant differences were observed in atrial α1A- and β1-AR and in plasma catecholamine levels between groups. Even though murine leptin produces dose-dependent increases in sympathetic discharge from the kidney and brown adipose tissue (15), plasma catecholamine may not be changed. These data suggest that basal ANP secretion, atrial AR mRNA, and isoproterenol-induced suppression of ANP secretion are not impaired under

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Fig. 6. Changes in plasma (P) catecholamine levels (A and B), and left atrial α1A- and β1-adrenergic receptor (AR) mRNA levels (C and D) during the first week in rats that received virus and PF rats. Experimental conditions are the same as in Fig. 4. EP, epinephrine; NE, norepinephrine. Values are means ± SE.

Fig. 7. Direct effects of leptin on pulse pressure (A), ECF translocation (B), ANP secretion (C) and concentration (D), and cAMP amount (E) in perfusate in isolated, perfused atria from control rats. After stabilization, atrial perfusate was collected at 2-min intervals at 4°C for 10 min, leptin (20, 100, 500 nM) was then introduced into the atrial lumen, and perfusates were continuously collected. Values are expressed as relative percent changes in atrial parameters between the five control values and the three peak experimental values (fraction 23–25) exposed to leptin. Experimental conditions are the same as in Fig. 5. Values are means ± SE. *P < 0.05 and **P < 0.01 vs. control group.
hyperleptinemic conditions. In in vitro experiments, high dose of leptin (100 and 500 nM) directly increased ANP secretion in isolated, perfused rat atria, but low dose of leptin (20 nM) did not. In our in vivo study, plasma leptin level was ~70 ng/ml, which equals ~7 nM. Therefore, based on the above data, we suggest that leptin decreases plasma ANP level indirectly.

Three receptor subtypes for natriuretic peptides, NPR-A, NPR-B, and NPR-C, have been identified, and their cDNA cloned (7,18). NPR-A and NPR-B are membrane-bound guanylate cyclases that mediate most of the biological actions of ANP, whereas NPR-C lacks the intracellular guanylate cyclase domain and mediates the cellular internalization and subsequent lysosomal degradation of ANP (18). If NPR-C is increased under hyperleptinemic condition, plasma ANP level will be decreased. Therefore, we addressed a possibility that characteristics of ANP receptors or their expression may be changed in hyperleptinemic rats. The results showed that the binding affinity and density of the ANP receptors for ANP and C-ANP were not altered, showing similar results with the control groups. These finding suggest that expression of ANP receptor and their characteristics are intact in hyperleptinemic rats.

Studies have been shown that acute hyperleptinemia induced by high doses of leptin (total dose, 100–1,000 μg/kg) does not affect BP (15,30). In the present study, acute injection of rat leptin (total dose, 290 μg/kg) in anesthetized rats increased BP by 20 mmHg without changing of HR, and an increase in BP was blocked by pretreatment with reserpine or l-NAME. Co-infusion of ANP with leptin did not cause leptin-induced increase in BP. These data suggest that acute administration of leptin increases BP by decreasing plasma ANP via NO, and that ANP may partly play an important function in leptin-induced hypertension. Our acute experimental protocol is similar to that of Haynes et al. (15) and others (30). The differences were the source of leptin (human leptin vs. rat leptin), amounts of dose, and anesthetics. However, the experimental outcomes did not agree with each other. Although the reasons for obtaining variable results among studies are not clearly understood, they may be depending on experimental duration, infusion route, and the condition of the animal. The source of leptin seems to affect experimental outcomes (16). Therefore, further studies are awaiting.

Chronic studies with leptin have also demonstrated that intravenous infusion of murine leptin (1 μg·kg⁻¹·min⁻¹) for 7 days resulted in an increase in BP and HR by 6 mmHg and 23 beats/min, respectively, while decreasing food intake by 50% (6,25). A study has reported that hyperleptinemic rats received leptin (twice a day at 250 μg/kg subcutaneously) for 7 days and shows an increased BP up to 25 mmHg with 15% decrease in food and water intake (3). In addition, transgenic mice over-expressing leptin resulted in an increase in BP of ~15–20 mmHg compared with control mice (2). In our experiments, hyperleptinemic rats having 70 ng/ml plasma leptin showed a transient increase in BP by 10 mmHg and a marked decrease in food intake by 40%. Our chronic results are similar to those of Carlyle et al. (6) in view of plasma leptin level, a decreased appetite, and direct measurement of BP, although the difference is the peak time of increased BP and changes in HR induced by leptin. In our in vivo studies, even though plasma ANP level decreased and PRC increased for 2 wk during hyperleptinemic conditions, BP increased transiently for 2 days and returned to control level. The reasons for the brief, small increase of BP in hyperleptinemic rats are not clearly understood at the present time. Interestingly, hyperleptinemic rats appear to retain sodium more than control rats, even though there was no statistically significant difference between paired animals and animals overexpressing leptin. Taken together, the transient increase in BP in hyperleptinemic rats may be due to a counterbalance between pressor and depressor mechanisms (such as NO) provided by leptin (19).

In summary, leptin decreases the plasma level of ANP via its indirect action on the NO system. The chronic effect of leptin on plasma ANP level is independent of atrial ANP content, ANP receptors in the kidney, or basal ANP secretion. So, we concluded that leptin might be an important regulator of ANP. From our data showing blockade of leptin-dependent hypertension by coinfusion of ANP in acute experiments, we speculate that leptin-induced decrease in ANP and increase in PRC could contribute to hypertension induced by leptin treatment or overexpression. However, an increase in BP by chronic exposure of leptin was transient, modest, and temporally not related to changes in ANP plasma levels. Further studies are needed to define compensatory systems that may contribute to maintain a constant BP under chronic hyperleptinemic condition.

REFERENCES


