Leptin Causes Vasodilation in Humans

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Leptin, a product of the ob gene, plays an important role in the regulation of body fat and has been suggested to cause vasodilation in rats. The purpose of this study was to evaluate whether leptin also has a vasodilating effect in humans. Using a strain-gauge plethysmography, we evaluated forearm blood flow (FBF) during intra-arterial infusion of leptin (1, 10 or 100 ng/kg/min for 5 min) in the absence and presence of the nitric oxide synthase inhibitor N(G)-monomethyl-L-arginine (L-NMMA; 8 μmol/min for 5 min) in ten healthy men (mean age, 23.0 ± 1.2 years). Leptin infusion significantly increased the FBF (8.5 ± 3.8, 20.3 ± 7.0 and 17.7 ± 5.4% at 1, 10 and 100 ng/kg/min of leptin, respectively; p < 0.05) and the forearm vascular resistance (FVR; -6.9 ± 3.1, -14.6 ± 4.3 and -13.4 ± 3.9% at 1, 10 and 100 ng/kg/min of leptin, respectively; p < 0.05). No significant changes in blood pressure or heart rate were detected during infusion of leptin. The intra-arterial infusion of L-NMMA did not alter the FBF response (6.6 ± 4.9, 22.1 ± 7.5, 13.3 ± 3.2% at 1, 10 and 100 ng/kg/min of leptin, respectively) or the FVR response (-4.3 ± 4.6, -15.2 ± 5.4, -11.1 ± 2.5% at 1, 10 and 100 ng/kg/min of leptin, respectively) to leptin. These findings suggest that leptin per se directly causes vasodilation and that leptin-induced vasodilatation is nitric oxide-independent in healthy men. (Hypertens Res 2002; 25: 161–165)

Key Words: leptin, nitric oxide, N(G)-monomethyl-L-arginine, endothelial function, plethysmography

Introduction

Obesity is one of the risk factors for systemic hypertension. Leptin, which is a peptide produced from the ob gene, is a circulating hormone secreted primarily by adipocytes (1). Leptin is increased in obese animals and humans, in correlation with body mass index or the amount of body fat (2, 3), and in pregnant women (4). Genetic deficiency of leptin and its receptors causes severe obesity in mice (1, 5) and humans (6, 7). Continuous infusion of recombinant leptin has been shown to decrease body weight and body fat by causing a decrease in food intake and increase in energy expenditure (8, 9). Interestingly, leptin shows a strong dose-dependent sympathoexcitatory effect, and this effect is mediated by leptin receptors in the hypothalamus. Acute i.v. leptin infusion did not affect arterial pressure or heart rate (10), whereas chronic leptin infusion significantly increased both (11). It has recently been shown that leptin promotes angiogenesis, and that long-form leptin receptors are expressed in the endothelium and are coupled to the JAKs-STAT signaling pathway (12). In addition, leptin has been reported to cause nitric oxide (NO)-induced vasodilation in Wistar Kyoto rats aorta (13). These findings suggest that the endothelium is a target of leptin. However, there is no information regarding the effect of leptin on the vasculature in humans.

To determine whether leptin induces vasodilation in the forearm circulation of humans, we performed intra-arterial infusion of leptin in the presence and absence of N(G)-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthase.
Methods

Subjects

The subjects were 10 healthy Japanese men (mean age, 23.0 ± 1.2 years). All of the subjects were non-smokers. Normal tension was defined as systolic pressure of less than 140 mmHg and diastolic blood pressure of less than 80 mmHg. The study protocol was approved by the ethical committee of Hiroshima University Faculty of Medicine. Informed consent for participation was obtained from all subjects.

Measurement of Forearm Blood Flow

Forearm blood flow (FBF) was measured by using a mercury-filled Silastic strain-gauge plethysmograph (EC-5R; D.E. Hokanson, Inc., Bellevue, USA) as previously described (14–16). Forearm vascular resistance (FVR) was calculated as the mean arterial pressure divided by FBF. Briefly, a strain-gauge was attached to the upper part of the left arm, connected to a plethysmography device, and supported above the right atrium. A wrist cuff was inflated to a pressure of 50 mmHg above the systolic pressure to exclude hand circulation for 1 min before each measurement and throughout the measurement of FBF. The upper arm congesting cuff was inflated to 40 mmHg for 7 s in each 15-s cycle to occlude venous outflow from the arm by using a rapid cuff inflator (EC-20; D.E. Hokanson, Inc.). The FBF output signal was transmitted to a recorder (U-228; Advance Co., Tokyo, Japan). FBF was expressed in ml/min/100 ml of forearm tissue volume. Four plethysmographic measurements were averaged at baseline for analysis of FBF during the administration of drugs. FBF was calculated from the linear portions of the plethysmographic recordings by two observers who were blinded to the results. The intraobserver coefficient of variation was 3.0 ± 1.8%.

Study Protocol

Throughout the study, subjects were kept in the supine position in a quiet, dark room maintained at 22°C to 25°C. A 23-gauge polyethylene catheter (Hakkow Co., Okayama, Japan) was inserted into the left brachial artery for the infusion of leptin and L-NMMA under local anesthesia (1% lidocaine), and a AP-641G pressure transducer (Nihon Kohden Co., Tokyo, Japan) was used for the recording of arterial pressure. Another catheter was inserted into the left deep antece- bital vein to obtain blood samples.

After 30 min in the supine position, saline and leptin (1, 10 and 100 ng/kg/min) were infused intra-arterially for 5 min using a constant rate infusion pump (Terfussion STG-523, Terumo Co., Tokyo, Japan). Then FBF and blood pressure during the last 2 min of infusion were measured. A blood sample was obtained from the deep antecubital vein as soon as the measurement of saline and the 100 ng/kg/min leptin infusion were finished.

After 1 h in the supine position, L-NMMA, an inhibitor of NO synthase, was infused intra-arterially at a dose of 8 µmol/min for 5 min. Then infusion of the same dose of saline and leptin were repeated to examine whether the effects of leptin on forearm hemodynamics were due to the contribution of leptin to the release of NO.

Baseline fasting serum concentrations of total cholesterol, high density lipoprotein cholesterol (HDL cholesterol), triglycerides, creatinine, insulin, glucose, electrolytes, and plasma renin activity (PRA), plasma angiotensin concentration (PAC), plasma concentrations of angiotensin II, epinephrine and norepinephrine were obtained after a 30-min rest period before the study.

Drugs

Human leptin and L-NMMA were obtained from Sigma Chemical Co. (St. Louis, USA). All other drugs were obtained from commercially available sources and were dissolved in saline (0.9% NaCl; Ohtsuka Pharmaceutical Co., Tokyo, Japan) immediately before use. All drugs were sterilized by specialists in the Department of Pharmacology, Hiroshima University Hospital.

Analytical Methods

Samples of venous blood were placed in polystyrene tubes containing EDTA-Na (1 mg/ml). The EDTA-containing tubes were chilled promptly in an ice bath. Plasma was separated immediately from the samples in EDTA-containing tubes by centrifugation at 3,100 g for 10 min at 4°C and serum at 1,000 g for 10 min at room temperature. Samples were stored at - 80°C until assayed. Routine chemical methods were used to determine serum concentrations of total cholesterol, HDL cholesterol, triglycerides, creatinine, and electrolytes. Serum glucose (Glucose Test, Kanto Chemical Co., Tokyo, Japan) was assayed by enzymatic method, and serum insulin (Phadeseph Insulin RIA Kit, Pharmacia Co., North Peapack, USA) was assayed by radioimmunoassay. The intra- and interassay coefficients of variation were 1.2 ± 0.4% and 0.6 ± 0.2% for glucose, and 6.8 ± 0.3% and 5.7 ± 1.6% for insulin. The serum concentration of low density lipoprotein (LDL) was estimated by using Friedwald’s method (17). Plasma epinephrine and norepinephrine (SRL Co., Tokyo, Japan) were assayed by high-performance liquid chromatography (HPLC). PRA (Gamma Coat PRA; SRL Co.), PAC (SPAC-S Aldosterone Kits; SRL Co.), plasma angiotensin II (angiotensin II antibody; SRL Co.) and plasma leptin (Human Leptin RIA Kit, Linco Research Inc., St. Charles, USA) were assayed by radioimmunoassay. The limit of sensitivity for the human leptin assay was 0.5 ng/ml, and the intra- and interassay coefficients of variation of the human leptin assay were 5.0 ± 0.9% and 4.5 ± 0.6%.
Table 1. Baseline Clinical Characteristics of the Subjects (n = 10)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
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<tbody>
<tr>
<td>Body weight (kg)</td>
<td>61.5 ± 1.9</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>21.3 ± 0.6</td>
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<tr>
<td>Mean blood pressure (mmHg)</td>
<td>86.8 ± 1.9</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>65.1 ± 3.0</td>
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<tr>
<td>FBF (ml/min/100 ml tissue)</td>
<td>6.3 ± 0.7</td>
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<tr>
<td>FVR (mmHg/ml/min/100 ml tissue)</td>
<td>15.6 ± 1.9</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.27 ± 0.18</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>0.81 ± 0.12</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.57 ± 0.07</td>
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<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.32 ± 0.20</td>
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<tr>
<td>Serum glucose (mmol/l)</td>
<td>4.9 ± 0.2</td>
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<tr>
<td>Serum insulin (pmol/l)</td>
<td>46 ± 8</td>
</tr>
<tr>
<td>PRA (ng/l/s)</td>
<td>0.48 ± 0.06</td>
</tr>
<tr>
<td>PAC (pg/ml)</td>
<td>105.8 ± 8.5</td>
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<tr>
<td>Ang II (pg/ml)</td>
<td>7.8 ± 1.2</td>
</tr>
<tr>
<td>Plasma epinephrine (pmol/l)</td>
<td>160 ± 20</td>
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<tr>
<td>Plasma norepinephrine (pmol/l)</td>
<td>1,280 ± 170</td>
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HDL, high density lipoprotein; LDL, low density lipoprotein; PRA, plasma renin activity; PAC, plasma aldosterone concentration; Ang II, angiotensin II; FBF, forearm blood flow; FVR, forearm vascular resistance. All results are presented as the mean ± SE.

Statistical Analysis

Results are presented as the mean ± SE. Values of p < 0.05 were considered to indicate statistical significance. Comparisons before and after drug infusion were analyzed by Wilcoxon’s paired test. Comparisons of dose-response curves of parameters during drug infusion were analyzed by repeated measures of ANOVA followed by post hoc testing with the least significant difference test. The data were processed using the software package Statistica 4.1 (Tulsa, USA).

Results

The baseline clinical characteristics of the 10 healthy male subjects are shown in Table 1. Plasma leptin levels in 5 subjects increased significantly with increasing doses of leptin in a dose-dependent manner (baseline, 2.62 ± 0.68 ng/ml; 1 ng/kg/min, 2.68 ± 0.64 ng/ml; 10 ng/kg/min, 3.28 ± 0.86 ng/ml; and 100 ng/kg/min, 40.88 ± 7.37 ng/ml, respectively). The concentration of leptin returned to the baseline level after a 30-min rest period. No significant change was observed in arterial blood pressure or heart rate by intra-arterial infusion of leptin (86.8 ± 1.9 to 85.4 ± 1.9 mmHg and 65.1 ± 3.0 to 66.8 ± 3.1 beats/min, respectively). Concentrations of plasma epinephrine and norepinephrine in the left deep ante-cubital vein did not change significantly between before and after leptin administration (160 ± 20 to 150 ± 20 pmol/l and 1,280 ± 170 to 1,030 ± 180 pmol/l, respectively). There were also no significant changes in serum glucose or insulin (4.9 ± 0.2 to 5.3 ± 0.3 mmol/l and 46 ± 8 to 72 ± 22 pmol/l, respectively).

The infusion of leptin caused a significant increase in FBF in the absence of L-NMMA (6.3 ± 0.7, 6.8 ± 0.8, 7.5 ± 0.8, and 7.4 ± 0.9 ml/min/100 ml tissue at baseline, 1, 10 and 100 ng/kg/min of leptin, respectively; p < 0.05 vs. baseline) and in the presence of L-NMMA (5.2 ± 0.6, 5.5 ± 0.7, 6.1 ± 0.6, and 5.8 ± 0.7 ml/min/100 ml tissue at baseline, 1, 10 and 100 ng/kg/min of leptin, respectively; p < 0.05 vs. baseline) (Fig. 1), and a decrease in FVR in the absence of L-NMMA (15.5 ± 1.9, 14.4 ± 1.7, 13.1 ± 1.6, and 13.4 ± 1.6 mmHg/ml/min/100 ml tissue at baseline, 1, 10 and 100 ng/kg/min of leptin, respectively; p < 0.05 vs. baseline) and in the presence of L-NMMA (18.5 ± 1.6, 17.6 ± 1.6, 15.3 ± 1.3, and 16.3 ± 1.4 mmHg/ml/min/100 ml tissue at baseline, 1, 10 and 100 ng/kg/min of leptin, respectively; p < 0.05, vs. baseline) (Fig. 2).
2), and all these changes were dose-dependent in all subjects. Intra-arterial infusion of the NO synthase inhibitor L-NMMA resulted in a significant decrease in basal FBF from 8.0 ± 1.0 to 5.2 ± 0.6 ml/min/100 ml tissue (p < 0.05) and an increase in basal FVR from 12.2 ± 1.4 to 18.5 ± 1.6 mmHg/ml/min/100 ml tissue (p < 0.05). No significant changes in arterial blood pressure and heart rate were observed during the infusion of L-NMMA (85.4 ± 1.9 to 86.9 ± 2.3 mmHg and 66.8 ± 3.1 to 63.5 ± 2.9 beats/min, respectively). The forearm vascular response to leptin was not modified by L-NMMA (Figs. 3 and 4).

**Discussion**

The results of this study suggest that leptin causes significant vasodilation in the forearm arteries of healthy humans and that this vasodilatory effect is not due to an increase in the release of NO induced by a sub-physiological leptin level.

Although it has been postulated that leptin-induced sympathetic nervous activation is mainly due to the central nervous system, details of this signaling pathway and neuronetwork system are not known. Recently, several investigators have reported that leptin may act on blood via leptin receptors. Leptin increases sympathetic nervous activity to brown adipose tissue and to tissue of the kidneys, adrenals, and hind limbs (10). Shek et al. (11) reported that chronic intravenous infusion of leptin increased blood pressure in Sprague-Dawley rats. These findings suggest that leptin may contribute to elevation of the blood pressure through an increase in sympathetic nervous activation. Haynes et al. (10) reported that sympathetic activation was induced a few minutes after administration of leptin, since sympathetic activation of leptin is mediated by the central nerve system. However, in the present study, the plasma concentrations of norepinephrine as one of the indexes of the action of the sympathetic nervous system did not alter throughout the study.

FBF was significantly increased, but there were no changes in blood pressure, heart rate or plasma catecholamines. In the present study, therefore, the sympathetic nervous system may not have contributed to the increase in FBF.

Generally, the plasma leptin level has been found to be strongly correlated with body mass index in humans. Leptin levels are greater in obese individuals than lean individuals. Even in obese subjects, however, plasma leptin levels are maintained within the physiological range of 10–200 ng/ml (2, 4). Therefore, in the present study, the plasma leptin concentration seemed to be maintained within the physiological range throughout the leptin infusion. These findings suggest that a physiological-level concentration of leptin directly causes vasodilation.

There are several possible mechanisms by which leptin evokes vasodilation. First, it was recently established that endothelium is a target organ of leptin (12). And it has been suggested that leptin may increase NO release (18). Frühbeck reported that intravenous administration of leptin caused an increase in serum nitrite/nitrate concentration in Wistar rats, suggesting that exogenous leptin loading induced NO release (18). Sierra-Honigmann et al. (12) have demonstrated the presence of full-length, long-form leptin receptors that are coupled to the JAKs-STAT signaling pathway in cultured human umbilical vein endothelial cells. However, it is difficult to discuss this mechanism because little is known about the post JAK-STAT signaling pathway of leptin in the endothelium. Our present findings show that leptin caused vasodilation via an NO-independent pathway in forearm circulation.

Second, it is quite likely that vasorelaxation of leptin is caused by an NO-independent mechanism, such as EDHF or prostaglandins. Recently, Lembo et al. (13) reported that leptin caused vasodilation in resistance arteries of rats through...
an increase in EDHF release, although leptin-induced vasodilation in conduit arteries was due to an increase in NO release. We cannot rule out the possibility that these vasoactive agents may contribute to leptin-induced vasodilation in the resistance arteries.

Third, it is possible that a change in insulin sensitivity affected the vascular response. However, there is little evidence supporting this hypothesis. Sivitz et al. reported that leptin increased insulin sensitivity in normal rats, but that a significant change in insulin sensitivity did not occur until about 45 min after leptin administration (19). Moreover, there were no significant changes in serum glucose or plasma insulin concentrations during leptin administration in the present study.

Finally, there is a possibility that leptin induces vaso-relaxation by directly affecting vascular smooth muscle cells.

All subjects recruited had no obesity (body mass index: 19.1 to 25.0 kg/m²) and low plasma leptin concentrations (1.4 to 5.2 ng/ml). It is well known that plasma leptin concentrations are elevated significantly in obese subjects, suggesting that there is a state of leptin resistance in obesity (2, 20, 21). Some possible mechanisms underlying leptin resistance have been postulated. First, such resistance may be due to leptin receptor mutation (7). Second, it may be related to alterations in the leptin signaling that activates the JAKs-STAT pathway (22). Future studies of leptin in obese subjects with leptin resistance are awaited with great interest. It is expected that the effects of leptin on the vasculature of obese subjects will differ from the effects in lean subjects.

In conclusion, we have shown that leptin causes dilatation of human forearm arteries and that this leptin-induced vasodilatation is due to an NO-independent pathway.

Acknowledgements

The authors thank Dr. Shigeaki Arai and Masahiko Sakai for the preparation and purification of all drugs, and Yuko Omura for her secretarial assistance.

References