Metabolism
Clinical and Experimental

VOL 48, NO 5 MAY 1999

PRELIMINARY REPORT

Lack of Leptin Suppression in Response to Hypersecretion of Catecholamines in Pheochromocytoma Patients

Antje Böttner, Graeme Eisenhofer, David J. Torpy, Monika Ehrhart-Bornstein, Harry R. Keiser, George P. Chrousos, and Stefan R. Bornstein

Leptin is a major regulator of body weight and energy balance and is subject to a variety of regulatory inputs. From several previous studies, catecholamines have been suggested to exert an inhibitory influence on leptin production in animals. In the present study, we analyzed leptin levels in relation to catecholamine hypersecretion in 27 human pheochromocytoma patients. A 10-fold increase in circulating norepinephrine ($P < .0001$) did not result in suppression of plasma leptin in the patients compared with normal controls (median and interquartile range, 4.3 ng/mL [2.4 to 6.8] *v* 2.2 ng/mL [1.9 to 3.0] in men and 18.6 [12.3 to 27.0] *v* 11.4 [10.1 to 15.9] in women). Correlation analysis indicated a significant association of leptin with epinephrine in normal subjects ($r = -.81$, $P < .0001$), but not in pheochromocytoma patients. Leptin was not related to norepinephrine in either group. In conclusion, our data suggest that a chronic elevation of catecholamines does not cause suppression of leptin secretion in patients with pheochromocytoma. This lack of effect may be attributable to the development of tolerance of adipose tissue leptin production to catecholamines.

THE ADIPOSTATIC HORMONE LEPTIN plays a pivotal role in maintaining energy balance and body weight.[1] The mechanisms that control leptin secretion in humans are incompletely understood, and various factors have been proposed to regulate leptin production independently of the adipose tissue mass.[2,3]

In animals, catecholamines and β-agonists reduce *ob* mRNA expression and leptin secretion in vitro[4-6] and in vivo,[7] suggesting that the sympathetic nervous system exerts an inhibitory effect on leptin production. In humans, administration of β-agonists has also been reported to reduce circulating leptin levels acutely.[8,9] However, whether endogenous catecholamines play a similar role in the regulation of leptin secretion in humans remains unclear.

Therefore, the objective of the present study was to evaluate leptin levels in relation to catecholamine hypersecretion as occurs in pheochromocytoma, and thus to evaluate the role of chronically elevated circulating catecholamines in the regulation of leptin production in humans.

SUBJECTS AND METHODS

The subjects included 16 male and 11 female pheochromocytoma patients and 10 normal control subjects matched for sex, age, weight, and body mass index (BMI). In the pheochromocytoma patients, the mean age was 39.6 ± 3.6 and 35.0 ± 11.6 years for men and women, respectively. The mean BMI was 25.04 ± 0.9 and 26.8 ± 5.3 kg/m^2 for male and female patients, respectively. Eight male patients were receiving α-blockers (phentolamine or prazosin). Ten healthy men (age, 36.4 ± 3.3 years; BMI, 23.87 ± 1.6 kg/m^2) and 10 healthy women (age, 32.6 ± 8.4 years; BMI, 26.25 ± 3.1 kg/m^2) served as controls. They were not taking any medication and were in good health as determined by a negative medical history and a normal physical examination. The study was approved by the Intramural Research Board. All subjects provided informed written consent for the study protocol.

From the Department of Internal Medicine III, University of Leipzig, Leipzig, Germany; Intramural Research Program, National Institute of Neurological Disorders and Stroke, Bethesda; Hypertension Endocrine Branch, National Heart, Lung, and Blood Institute, Bethesda; Developmental Endocrinology Branch, National Institute of Child Health and Human Development, Bethesda, MD; and Queen Elizabeth Hospital, University of Adelaide, Adelaide, Australia.

Submitted August 21, 1998; accepted December 21, 1998.

Supported by a grant from the Boehringer Ingelheim Fond (A.B.), a grant from the Wilhelm Sander Stiftung (95.033.1 to M.E.-B.), and grants from the Deutsche Forschungsgemeinschaft (Heisenberg grant no. DFG BO1141/6-1 and SFB351/C8 to S.R.B.).

Address reprint requests to Antje Böttner, MD, University of Leipzig, Department of Internal Medicine III, Ph.-Rosenthal-Str.27, 04103 Leipzig, Germany. Email: med93cvj@studserv.uni-leipzig.de.

0026-0495/99/4805-0001$10.00/0

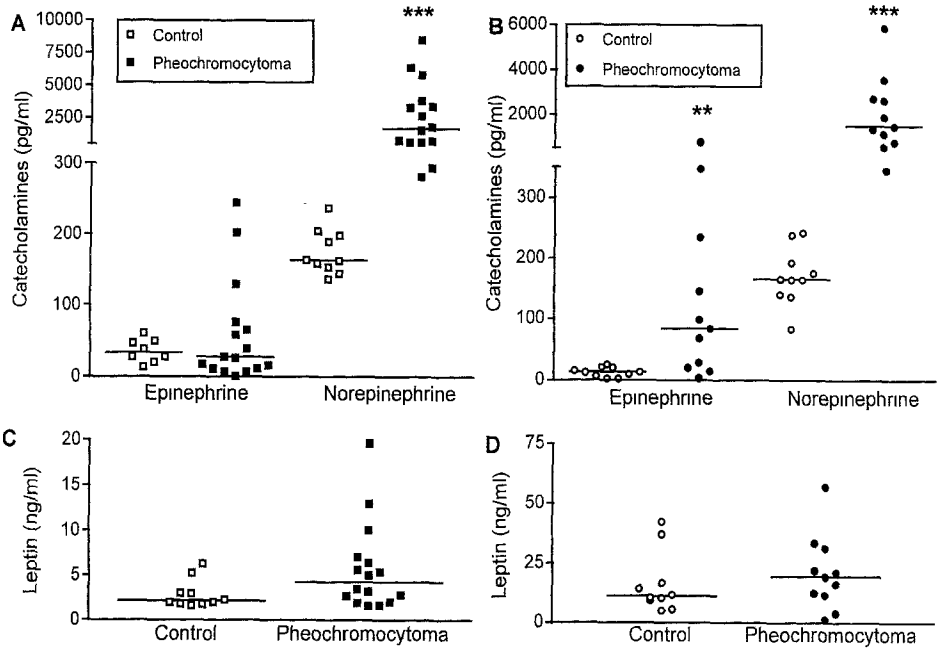

Fig 1. Plasma catecholamines and leptin in male (A, C) and female (B, D) pheochromocytoma patients and control subjects. Horizontal lines indicate the median. Significance was calculated with the Mann-Whitney test: ***$P < .0001$; **$P < .01$.

For blood collection, subjects were instructed to eat a light breakfast and were studied at approximately the same time in the morning. An indwelling intravenous cannula was implanted, and the subjects rested quietly for at least 15 minutes before blood sampling. Plasma catecholamines were determined by liquid chromatography with electrochemical detection after a batch alumina extraction as previously described in detail.[10] Leptin levels were determined using a commercially available leptin radioimmunoassay kit (DRG Instruments, Marburg, Germany). The assay sensitivity was 0.5 ng/mL and the intraassay and interassay coefficients of variation were 8.3% and 6.2%, respectively.

Data were analyzed using the Mann-Whitney test and nonparametric correlation analysis (Spearman). Results are expressed as the medians and interquartile range. Unless stated otherwise, statistical significance was defined as a P level less than .05.

RESULTS

The plasma norepinephrine level was 10-fold higher in pheochromocytoma patients versus normal controls ($P < .0001$, n = 10). In contrast, circulating epinephrine was not dramatically increased in male patients, whereas female patients exhibited significantly elevated epinephrine levels. Compared with normal control subjects, leptin levels were not significantly changed in either male patients (4.3 ng/mL [2.4 to 6.8] *v* 2.2 ng/mL [1.9 to 3.0]) or female patients (18.6 ng/mL [12.3 to 27.0] *v* 11.4 ng/mL [10.1 to 15.9]) (Fig 1). Independently of the BMI, no differences were detectable between pheochromocytoma patients treated with α-blockers and untreated patients (5.3 ng/mL [2.7 to 6.1] *v* 3.2 ng/mL [2.4 to 8.6]).

Leptin correlated strongly with the BMI and body weight in male and female pheochromocytoma patients ($r = .66$ and $r = .65$, respectively, $P < .05$) and healthy controls ($r = .64$, $P < .05$). In normal male controls, a significant negative correlation between leptin and epinephrine was determined ($r = -.81$, $P < .0001$) (Fig 2), which remained after leptin levels were adjusted for the BMI. No such correlation was evident in pheochromocytoma patients, and there was no relation between norepinephrine and leptin in any group.

In all comparative and correlation analyses, adjustment of leptin for the BMI did not affect the results presented.

DISCUSSION

Overt hypersecretion of catecholamines did not result in suppression of circulating leptin levels in human pheochromocytoma patients, who presented with plasma leptin levels similar to those reported elsewhere for normal lean human subjects.[11] This is in contrast to what might be expected from previous studies proposing the presence of a negative feedback loop by which catecholamines inhibit leptin production.[12]

This concept mainly derives from animal studies, which may,

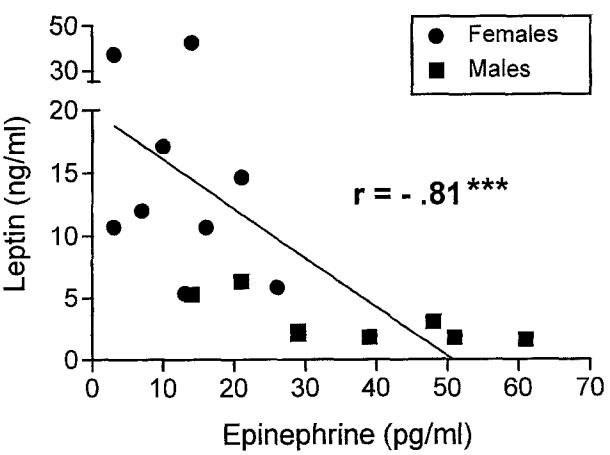

Fig 2. Association between leptin and epinephrine in normal healthy subjects. The correlation was determined by nonparametric correlation analysis (Spearman). ***$P < .0001$.

however, not accurately reflect the situation in vivo in human subjects. In this regard, differences in body fat distribution, as well as the varying physiological importance of adrenergic receptors in the regulation of adipose tissue function according to species, sex, and anatomic location of fat deposits, must be considered.[13]

Studies investigating the impact of catecholamines on leptin regulation in humans are scarce and have not yet presented conclusive evidence. Contrary to our results, there is a recent report describing low leptin in a 72-year-old patient with pheochromocytoma, which increased after curative surgery.[14] In normal human subjects, plasma leptin was observed to decline rapidly in response to infusion of β-agonists.[8,9] In contrast, a state of experimentally decreased norepinephrine did not induce significant alterations in leptin production in another study.[15]

Most previous studies investigated the impact of short-term administration of norepinephrine or β-agonists on leptin production, and have not considered the effect of sustained hypercatecholaminemia. The lack of leptin suppression in states of chronically elevated catecholamines, as shown in our patients, may well be attributable to the downregulation of functional adrenergic receptors. Supporting this contention, in a recent study, long-term stimulation with norepinephrine resulted in a complete desensitization of the β-adrenergic response in adipocytes from a patient with pheochromocytoma and in a human adipocyte cell line.[16] However, the absence of an effect of hypercatecholaminemia may not generalize to a lack of effect of catecholamines on leptin production, since the transmitter concentration in the synaptic cleft could be much higher than the plasma levels measured. As expected, and consistent with the well-established fact that leptin is most highly correlated with parameters of body fat, the association between leptin and the BMI was preserved in the pheochromocytoma patients. However, this does not generally exclude additional regulatory influences that account for the variation in leptin levels in human subjects.

Whereas the effect of norepinephrine and specific β-adrenergic agonists on leptin production has been repeatedly studied, the role of epinephrine in this regulatory process is not documented. The two major catecholamines are known to operate differentially to regulate several metabolic functions on the basis of their distinct affinity for adrenergic receptors and the local distribution of receptor subtypes in the tissue.[17] The inhibitory effect of catecholamines on leptin production has been shown to be mediated via β_3 receptors,[18] which are supposedly more sensitive to epinephrine than norepinephrine.[19] In accordance with this concept, our results indicate that in normal men, epinephrine displays a significant negative correlation with leptin, whereas norepinephrine does not.

In summary, despite a marked oversecretion of norepinephrine, circulating leptin levels were not suppressed in human pheochromocytoma patients. This suggests that leptin is not under inhibitory adrenergic control in humans with chronic hypercatecholaminemia. Downregulation of adipose tissue adrenergic receptors may explain this phenomenon.

REFERENCES

1. Bray GA, York DA: Leptin and clinical medicine: A new piece in the puzzle of obesity. J Clin Endocrinol Metab 82:2771-2776, 1997
2. Sinha MK, Caro JF: Clinical aspects of leptin. Vitam Horm 54.1-30, 1998
3. Orban Z, Bornstein SR, Chrousos GP: The interaction between leptin and the hypothalamic-pituitary-thyroid axis. Horm Metab Res 30:231-235, 1998
4. Kosaki A, Yamada K, Kuzuya H: Reduced expression of the leptin gene (ob) by catecholamine through a G(S) protein–coupled pathway in 3T3-L1 adipocytes. Diabetes 45:1744-1749, 1996
5. Hardie LJ, Guilhot N, Trayhurn P. Regulation of leptin production in cultured mature white adipocytes. Horm Metab Res 28:685-689, 1996
6. Gettys TW, Harkness PJ, Watson PM: The beta 3-adrenergic receptor inhibits insulin-stimulated leptin secretion from isolated rat adipocytes. Endocrinology 137:4054-4057, 1996
7. Trayhurn P, Duncan JS, Rayner DV, et al: Rapid inhibition of ob gene expression and circulating leptin levels in lean mice by the beta 3-adrenoceptor agonists BRL 35135A and ZD2079. Biochem Biophys Res Commun 228:605-610, 1996
8. Pinkney JH, Coppack SW, Mohamed-Ali V: Effect of isoprenaline on plasma leptin and lipolysis in humans Clin Endocrinol (Oxf) 48:407-411, 1998
9. Donahoo WT, Jensen DR, Yost TJ, et al: Isoproterenol and somatostatin decrease plasma leptin in humans: A novel mechanism regulating leptin secretion. J Clin Endocrinol Metab 82:4139-4143, 1998
10. Holmes C, Eisenhofer G, Goldstein DS: Improved assay for plasma dihydroxyphenyl-acetic acid and other catechols using high-performance liquid chromatography with electrochemical detection. J Chromatogr 653:131-138, 1994
11. Considine RV, Sinha MK, Heiman ML, et al: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292-295, 1996
12. Giacobino J-P: Role of the β_3-adrenoceptor in the control of leptin expression. Horm Metab Res 28:633-637, 1996
13. Lafontan M, Bousquet-Melou A, Galitzky J, et al: Adrenergic receptors and fat cells: Differential recruitment by physiological amines and homologous regulation. Obes Res 3:507S-514S, 1995
14. Sakane N, Yoshida T, Mizutani T, et al: Serum leptin levels in a patient with pheochromocytoma. J Clin Endocrinol Metab 83:1400, 1998 (letter)
15. Zimmermann RC, Krahn L, Rahmanie N, et al: Prolonged inhibition of presynaptic catecholamine synthesis does not alter leptin secretion in normal-weight men and women. Hum Reprod 13:822-825, 1998
16. Jockers R, Issad T, Zilberfarb V, et al: Desensitization of the β-adrenergic response in human brown adipocytes. Endocrinology 139:2676-2684, 1998
17. Young JB, Rosa RM, Landsberg L: Dissociation of sympathetic nervous system and adrenal medullary responses. Am J Physiol 247:E35-E40, 1984
18. Deng C, Moinat M, Curtis L, et al: Effects of β-adrenoceptor subtype stimulation on obese gene messenger ribonucleid acid and on leptin secretion in mouse brown adipocytes differentiated in culture. Endocrinology 138:548-552, 1997
19. Arch JRS, Wilson S: β_3-Adrenoceptors and the regulation of metabolism in adipose tissues Biochem Soc Trans 24:412-418, 1996