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ORIGINAL ARTICLE

Cerebrospinal fluid xenin levels during body mass reduction: no evidence for obesity-associated defective transport across the blood–brain barrier

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CONTEXT: Recent studies have shown that xenin can act in the hypothalamus, reducing food intake through a leptin- and melanocortin system-independent mechanism.
OBJECTIVE: To evaluate the impact of body mass reduction on the blood and cerebrospinal fluid (CSF) levels of xenin.
DESIGN AND SETTING: Thirteen obese patients (11 women) selected for roux-in-Y gastric bypass surgery were evaluated before and approximately 8 months after surgery. Xenin was determined in serum and CSF by radioimmunoassay.
RESULTS: As compared with lean subjects, obese patients have increased blood levels of xenin, which reduce after surgery. There are significant correlations between blood xenin and blood leptin and insulin levels. CSF concentration of xenin is ~10-fold lower than blood levels, and is significantly higher in obese subjects as compared with lean ones, returning to normal levels after body mass reduction. There is a significant linear correlation between CSF and blood levels of xenin.
CONCLUSION: Xenin is present in the human CSF in a concentration ~10-fold lower than the blood. Both blood and CSF xenin are correlated with blood levels of important markers of adiposity, leptin and insulin. The levels of CSF xenin are linearly correlated with blood xenin, independently of patient body mass, suggesting that either its transport across the blood–brain barrier is not saturated in the concentration range detected in this study or that there is a coordinated release of xenin from the periphery and the CNS.

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INTRODUCTION

The neurotensin-like hormone, xenin, is a 25-amino-acid peptide (molecular mass 2971) produced by the gastric mucosa, and is secreted predominantly after meals.[1,2] Initial reports provide evidence for a role of xenin in the control of exocrine pancreatic function.[1] However, recent studies have shown that xenin produces a potent anorexigenic effect[3–6] by acting in the hypothalamus independently of leptin and of the AgRP/melanocortin system.[5]

Leptin provides the most robust adipostatic signal to the hypothalamus.[7] It acts predominantly in the arcuate nucleus, inhibiting NPY/AgRP while stimulating POMC neurons.[7] Studies conducted right after leptin identification frustrated the initial expectations regarding its potential therapeutic use in obesity.[8,9] In fact, obese patients present a defective transport of leptin through the blood–brain barrier (BBB),[10,11] and animal models of obesity are leptin resistant because of an impaired signal transduction.[12] Thus, it is expected that mechanisms capable of bypassing the leptin signal in the hypothalamus may be useful for treating obesity.

As xenin exerts a leptin-independent, central nervous system (CNS)-dependent anorexigenic action, we decided to evaluate the blood and cerebrospinal fluid (CSF) levels of this hormone in massively obese patients before and after body mass reduction provided by bariatric surgery. Our results suggest that, in contrast to leptin, xenin transport across the BBB is preserved in obesity.

PATIENTS AND METHODS

Patients (13, 11 females) were selected at the Clinics Hospital of the University of Campinas. Bariatric surgery was recommended according to the National Institutes of Health criteria.[13] Patients in the age group of 18–60 years were considered eligible for the study. Exclusion criteria were inflammatory or infectious diseases, diabetes mellitus, neurologic or psychiatric illnesses, alcohol consumption of more than 30/15 g per day for men and women, respectively, smoking and use of psychotropic or anti-inflammatory drugs. Patients were evaluated before and ~8 months after a Roux-in-Y gastric bypass, which was performed as previously described.[14] Anthropometric evaluation was performed at every visit. Lean subjects were selected among students of the University of Campinas, whereas CSF samples were obtained from lean subjects undergoing routine examinations, who revealed no illness after completion of the investigation.

Blood and CSF samples were collected after an overnight fast on the day of the surgery and on the final visit, ~8 months later. Insulin, leptin and adiponectin were evaluated in sera using ELISA kits from Millipore (Billerica, MA, USA). Xenin levels in both serum and CSF were determined using an RIA kit from Phoenix Pharmaceuticals (Burlingame, CA, USA). CSF and blood biochemical parameters were measured using automated

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methods from Beckman Coulter (Brea, CA, USA) and F Hoffmann-La Roche (Basel, Switzerland).

The study was approved by the University of Campinas Ethics Committee, and written informed consent was obtained from all patients. A two-tailed, paired *t*-test was used to evaluate changes from baseline conditions to those at 8 months. Xenin data in blood and CSF were analyzed using the Pearson's correlation coefficient. A $P<0.05$ was accepted as significant.

RESULTS

Approximately 8 months after surgery, significant reductions in body mass index and waist/hip ratio were accompanied by the reductions of the systemic markers of inflammation, C-reactive protein and erythrocyte sedimentation rate (Table 1).

Obese patients had a significantly higher mean serum xenin level than lean subjects and, after body mass reduction, the levels were normalized (Figure 1a); however, there were no significant correlations between these parameters. Nevertheless, significant correlations were observed between serum xenin and serum leptin and serum insulin, two important markers of adiposity (Table 2). No correlations were detected between serum xenin and serum adiponectin, serum IL10 and serum IL6 (Table 2).

Similar to serum xenin, in the CSF, the levels of the hormone, which are ~10-fold lower than serum levels, were significantly higher in obese subjects before surgery, returning to levels similar to those of lean subjects after body mass reduction (Figure 1b). Although CSF xenin was not correlated with BMI, it correlated significantly with other markers of adiposity, leptin (Figure 1d) and insulin (Figure 1e), and inversely correlated with adiponectin (Figure 1f). Interestingly, there was a significant linear correlation between serum and CSF xenin (Figure 1c). Finally, as in serum, CSF xenin was not correlated with CSF levels of the cytokines IL10 and IL6 (Table 3).

DISCUSSION

A number of peptides are produced by the gastrointestinal tract in response to the presence or, eventually, absence of food.[15] Most of these peptides are known to produce modulatory effects on food intake, and by several means have been tested for their potential use in obesity. Unfortunately, on most occasions, pharmacological or genetic manipulation of these systems results only in immediate changes in food intake, producing no long-term changes in body adiposity.[16] One exception is GLP1; both in experimental models and humans, the increased activity of this peptide results in some change in body mass. However, in patients using drugs that either reduce the activity of the GLP1-degrading enzyme, DPP-IV, or mimic the peptide's action, the resulting body mass variation is, at most, 5%.[17]

Table 1. General parameters of the subjects

	Obese BS	*Obese AS*	*Lean*
Gender (F/M)	11/2	11/2	6/2
Age (years)	34 ± 10	35 ± 10	29 ± 4
BMI (kg m^{-2})	39 ± 2	28 ± 3*	21 ± 2*
Waist/hip	0.86 ± 0.18	0.83 ± 0.12*	0.79 ± 0.09*
CRP (mg dl^{-1})	0.91 ± 0.70	0.17 ± 0.02*	0.13 ± 0.02*
ESR (mm h^{-1})	26 ± 16	16 ± 9*	14 ± 12*

Abbreviations: BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; F, female; M, male; obese BS, obese before surgery; obese AS, obese after surgery. Data are presented as mean ± s.d. *$P\leqslant0.05$ vs obese BS.

In general terms, by far the most robust result in body mass is obtained by modulation of the leptin signaling system.[7] Increases of more than 100% in adiposity are achieved in both humans and animals lacking leptin or a functional leptin receptor.[18] Most of the adipostatic effect of leptin in the hypothalamus depends on a functional melanocortin system.[16,18] This is supported by the fact that the most prevalent human monogenic defects leading to massive obesity are the mutations of the MC4R, one of the receptors for POMC.[18] As, in most cases of obesity, there is a resistance to the action of leptin, which results in insufficient activation of the melanocortin system, it is suspected that only approaches bypassing the leptin/melanocortin system will be efficient to treat obesity.

The recent demonstration of the leptin/melanocortin-bypassing properties of xenin boosted the interest in this peptide as a candidate approach to deal with obesity.[5] One important issue when dealing with CNS-acting peptides is their capacity to cross the BBB. As for leptin, defective transport across the BBB is regarded as one of the components of the resistance to this hormone in obesity.[10,11] Thus, we decided to evaluate the presence of xenin in the CNS of obese and lean subjects. For this, we evaluated a group of 13 massively obese patients undergoing bariatric surgery, and eight lean controls. Unlike leptin, serum and CSF xenin levels were significantly correlated in all subjects, suggesting that xenin transport across the BBB is preserved in obesity, and that this peptide enters the brain either by a non-saturable transport system, or that the threshold for saturation is higher than the blood concentration achieved in obesity. An alternative explanation for this linear correlation could

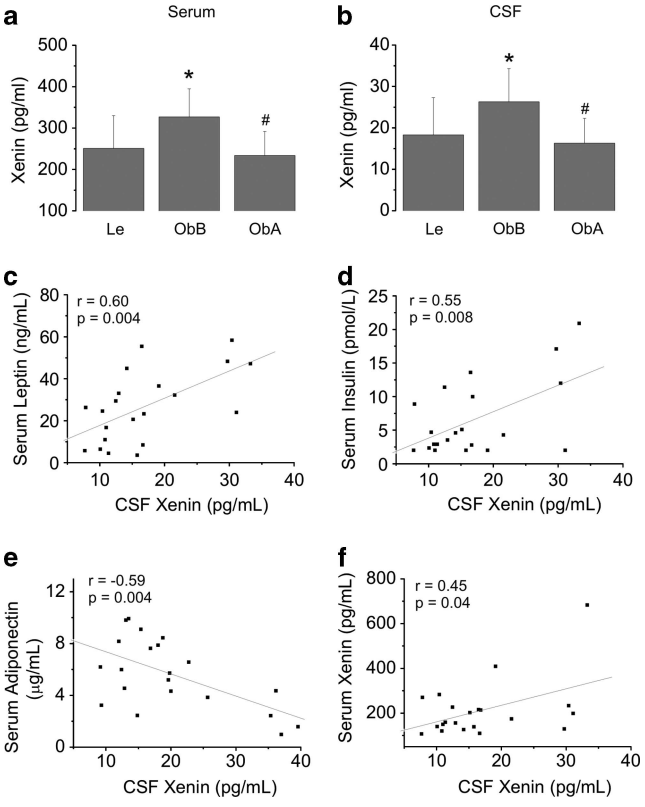

Figure 1. The mean concentrations of xenin in the serum and CSF of lean (Le), obese patients before surgery (ObB) and obese patients after surgery (ObA) are presented in (**a**) and (**b**), respectively. The graphic representation of the correlation between serum leptin (**c**), insulin (**d**), adiponectin (**e**) and xenin (**f**) vs CSF xenin are depicted. In all figures, lean controls = 8; obese patients = 13; in (**a**) and (**b**), *$P<0.05$ vs Le, $^{\#}P<0.05$ vs ObB.

Table 2. Correlation of serum xenin with BMI and serum hormones and cytokines

	r	P
BMI (kg m^{-2}) vsserum xenin (pg ml^{-1})		
Lean	−0.15	0.71
Obese BS	0.29	0.32
Obese AS	0.47	0.11
Lean + obese BS + obese AS	0.12	0.47
Serum xenin (pg ml^{-1}) vs serum leptin (ng ml^{-1})		
Lean	0.25	0.55
Obese BS	0.13	0.65
Obese AS	0.31	0.17
Lean + obese BS + obese AS	0.41	0.05*
Serum xenin (pg ml^{-1}) vs serum insulin (pmol l^{-1})		
Lean	0.71	0.04*
Obese BS	0.49	0.08
Obese AS	0.09	0.76
Lean + obese BS + obese AS	0.43	0.01*
Serum xenin (pg ml^{-1}) vs serum adiponectin (μg ml^{-1})		
Lean	−0.18	0.66
Obese BS	−0.03	0.91
Obese AS	0.09	0.76
Lean + obese BS + obese AS	−0.18	0.29
Serum xenin (pg ml^{-1}) vs serum IL10 (pg ml^{-1})		
Lean	−0.05	0.88
Obese BS	0.09	0.75
Obese AS	−0.18	0.54
Lean + obese BS + obese AS	0.01	0.93
Serum xenin (pg ml^{-1}) vs serum IL6 (pg ml^{-1})		
Lean	−0.08	0.83
Obese BS	−0.20	0.49
Obese AS	0.05	0.84
Lean + obese BS + obese AS	0.01	0.98

Abbreviations: BMI, body mass index; IL10, interleukin-10; IL6, interleukin-6; obese BS, obese before surgery; obese AS, obese after surgery. *$P \leqslant 0.05$.

Table 3. Correlation of CSF xenin with BMI and CSF cytokines

	r	P
BMI (kg m^{-2}) vs CSFxenin (pg ml^{-1})		
Lean	0.03	0.95
Obese BS	0.34	0.32
Obese AS	0.38	0.24
Lean + obese BS + obese AS	0.01	0.93
CSF xenin (pg ml^{-1}) vs CSF IL10 (pg ml^{-1})		
Lean	−0.01	0.97
Obese BS	−0.33	0.33
Obese AS	−0.18	0.58
Lean + obese BS + obese AS	0.01	0.93
CSF xenin (pg ml^{-1}) vs CSF IL6 (pg ml^{-1})		
Lean	0.53	0.35
Obese BS	0.49	0.14
Obese AS	0.30	0.36
Lean + obese BS + obese AS	0.20	0.31

Abbreviations: BMI, body mass index; CSF, cerebrospinal fluid; IL10, interleukin-10; IL6, interleukin-6; obese BS, obese before surgery; obese AS, obese after surgery.

be a coordinated release of xenin from the periphery and the CNS, as we cannot rule out a central source of the peptide. Recent studies have demonstrated that both central and peripheral administration of xenin to rodents[3,5,6] or chicks[4] results in reduction of food intake in a dose-dependent manner. Peripheral administration of xenin stimulates c-Fos expression in the hypothalamus[4,5] and nucleus of the solitary tract of the brainstem,[19] indicating that the peripherally produced hormone exerts its effects, at least in part, by a direct action on the central nervous system. The observation of a similar anorectic effect after xenin administration in animal models of obesity, including *ob/ob* and agouti mice,[5] suggested that xenin can alter feeding independently of leptin and the melanocortin signaling pathway, and that obesity in these models is not associated with resistance to xenin actions, raising the interest in xenin as a therapeutic option for obesity. However, one study demonstrated that chronic peripheral administration of xenin to mice had no significant effect on daily food intake or body weight.[6] The authors pointed out that this may be due to the relatively low doses used, and further studies are required to address the therapeutic potential of xenin.

Our results show that xenin levels are significantly correlated with systemic levels of known markers of adiposity, but not with BMI. The possible reasons for this are the relatively small size of the sample, or the fact that BMI does not reflect body adiposity accurately in all subjects.

Fasting xenin levels, both in serum and CSF, were significantly higher in obese subjects and decreased after surgery, reaching levels similar to those of lean subjects. Conversely, fasting serum levels of other satiety factors, including peptide YY (PYY) and GLP-1, are reduced in obesity and increase after surgery, in line with their action in promoting satiety.[20–22] The reasons for this discrepancy are unclear. These studies have also shown that postprandial release of these peptides is lower in obese subjects than in lean ones.[20–22] We did not evaluate the xenin response after food ingestion, and it is possible that this response is blunted in obesity, despite higher fasting levels.

In the present study, we have demonstrated for the first time the presence of xenin in the CSF of humans, and fasting serum and CSF levels of this peptide are increased in obesity and decrease after bariatric surgery-induced body mass loss. Importantly, xenin transport across the BBB seems preserved in obesity. Although xenin properties point to an interesting option for therapeutic purposes, its precise role in obesity and related diseases remains to be determined.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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