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Brain leptin resistance in human obesity revisited☆

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Abstract

Leptin is a 16 kDa peptide predominantly produced by adipocytes. Leptin and its receptor are known to be involved in the regulation of energy balance. The data from animal studies as well as our own observations of leptin overflow from the brain suggest that the central nervous system is a site of leptin synthesis. Using simultaneous arterio-venous blood sampling we here confirm that leptin is released from the brain into the internal jugular vein, and that release is greater in overweight men and in females compared to lean men, 467.3 ng/min±160.4 and 1426 ng/min±769.3 vs 80.0 ng/min±29.3, respectively ($P<0.05$). Furthermore, we have examined the gene expression of leptin and its receptor isoforms by reverse transcription-polymerase chain reaction (RT-PCR) in human cadaver hypothalami across a broad range of adiposity. Leptin gene expression was detected in a number of donors; the presence of detectable leptin mRNA was related to the mode of death rather than BMI or gender. We have also demonstrated gene expression of the three leptin receptor isoforms in the human hypothalamus. No relation was observed between the levels of hypothalamic expression of the long signaling form of the leptin receptor and BMI. In summary, this study indicates that it is very difficult to explain human obesity on the basis of central nervous system "leptin resistance", in that leptin is released in the brain, and at a higher level in the obese, and brain leptin receptor gene expression is not impaired in obesity.

Keywords: Leptin; Internal jugular vein; Leptin gene; Leptin receptor; Hypothalamus

1. Introduction

Leptin, the adipose tissue-derived hormone, plays an important role in regulating appetite and energy expenditure, by acting on specific receptors in the hypothalamus [1]. It is released by fat cells at levels proportional to body fat stores and plasma leptin levels strongly correlate with the degree of adiposity, with greater levels found in overweight individuals and reduced levels found in lipodystrophic animals. Obese humans and animals have elevated plasma leptin levels; therefore "leptin deficiency" is not the cause of most cases of obesity. Leptin resistance has been suggested as an explanation for why increased plasma leptin concentrations do not have the expected weight reducing effect.

A number of mechanisms have been proposed to explain leptin resistance. The transport of leptin into the central nervous system represents an important step in the regulation of body weight. It has been suggested that one basis for leptin resistance might be failure of leptin to cross the blood–brain barrier. Studies by Banks and Farrell and Schwartz et al. [2,3] do provide evidence of impaired transport of leptin into the central nervous system in obesity. Previous experimental studies were successful in demonstrating that central leptin administration, as opposed to peripheral, is more effective in reducing appetite and stimulating thermogenesis [4].

When first discovered, leptin was thought to be secreted into the bloodstream exclusively by adipocytes [5]. Later evidence, however, suggests that adipose tissue may not be the only site of leptin production, with demonstration of leptin gene expression in other tissues, including the stomach [6], placenta [7], and skeletal muscles [8]. Previous studies from our laboratory demonstrated a net efflux of leptin from the brain into the internal jugular veins [9–11]. These observations suggest that perhaps the brain itself produces leptin, which acts locally and is subsequently released into the circulation, contributing to the total leptin plasma pool

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[9]. In fact, leptin mRNA expression has been demonstrated in the rat pituitary and brain [12].

There are six leptin receptor isoforms, which are splicing variants of a single gene transcript [13,14]. Regions identical to all receptor isoforms include an extracellular domain, a transmembrane domain, and the first 29 amino acids of the cytoplasmic domain. Based on the length of the intracellular domain, one of the isoforms is "long" (OB-Rb), and four are "short". These differ in their cytosolic carboxy terminus. In addition, there is one soluble isoform (OB-Re), lacking the transmembrane domain, which may be involved in leptin transport from plasma [15]. When leptin binds to the full-length OB-Rb receptor, it can be demonstrated to activate JAK/STAT (Janus kinase/signal transducer and activator of transcription) and MAPK (mitogen activated protein kinase) signal transduction pathways in a variety of in vitro systems [16]. The first demonstration of brain leptin receptor gene expression came soon after the discovery of leptin [17]. Loss of function mutations, resulting in complete leptin resistance has been demonstrated to cause morbid obesity in some experimental models of rodent obesity [18,19]. Leptin receptor gene mutations, however, are very rare in humans [1].

Based on previous findings we set out to further investigate whether the human brain is a site of leptin production, and what might be the contribution of brain leptin to the plasma leptin pool. We also investigated the expression of leptin receptor isoforms in the hypothalamus and whether this was related to the degree of adiposity and to gender.

2. Methods

2.1. Experimental subjects

The subjects for the study were recruited from the general community. All participants had thorough clinical screening, which included clinical evaluation and serum biochemistry measurements to exclude hepatic and renal dysfunction. Experiments involved arterial and central venous catheterisation for neurochemical measurements. Dietary sodium intake and caloric intake in the recruited subjects were unrestricted at the time of the study. The defining characteristics of the subject groups recruited for the studies are outlined below in Table 1. Participation in the studies was only after written informed consent, with the approval of the Alfred Hospital Ethics Review Committee.

Respondents with a history of cardiovascular disease, diabetes, chronic medication, a blood pressure greater than 140/85 mm Hg or an alcohol intake of more than two standard drinks per day were excluded from the studies.

The degree of obesity of subjects was assessed using the body mass index (BMI). BMI is derived from the relation of weight to height squared (kg/m^2). Overweight but otherwise healthy participants with BMI greater than 28 were recruited for the study. All subjects had been weight stable (±1 kg) for at least two months prior to the study. Lean subjects did not have any prior history of obesity.

The age, gender, BMI and arterial leptin concentrations for these subjects are given in Table 1.

Table 1
Subject characteristics and arterial plasma leptin concentrations

	Lean males	Overweight males	Lean females
N	16	14	12
Age (years)	46.3±4.8	44.4±2.8	36.6±2.6
BMI (kg/m^2)	22.6±0.5	30.2±0.6*	22.2±0.7
Arterial plasma leptin concentration (ng/ml)	3.1±0.4	10.4±1.9*	10.9±2.0*

Mean±SEM.
* $P<0.001$ vs lean males.

2.2. Catheter procedure and blood sampling

The subjects were asked to not smoke or drink beverages containing caffeine and alcohol for at least 12 h before the catheter study. On the morning of the study, the subjects had a standardised light breakfast (350 kcal), typically comprising fruit juice and toast. The study was performed with the subjects at supine rest. A 21-gauge cannula (model C-PMS-301-RA 3.0F, 5 cm arterial catheter, Cook Australia, Eight Mile Plains, Queensland, Australia) was introduced percutaneously into the brachial or radial artery of either arm for arterial blood sampling and pressure monitoring.

Central venous catheterisation was performed under direct fluoroscopic control via an 8.5 French gauge introducer sheath (Arrow International Inc., Reading, Pennsylvania, USA) that was inserted percutaneously under local anaesthesia into the median antecubital vein. The internal jugular vein and renal vein were reached by maneuvering this single central catheter under direct fluoroscopic vision. Correct positioning of the catheter was confirmed using 2 ml radiopaque contrast medium (Omnipaque, Winthrop Pharmaceuticals, NY, USA). In the internal jugular vein, the catheter tip was above the angle of the jaw, to exclude sampling from the tissues of the face. Internal jugular blood flow was determined by thermodilution [20], and plasma flow after adjustment for the haematocrit. Renal plasma flow was calculated from the renal clearance of para-aminohippuric acid [21].

Blood samples for the measurement of leptin concentration were obtained simultaneously from the arterial and internal jugular venous catheters and immediately placed into ice-chilled tubes containing glutathione as antioxidant, and EGTA as anticoagulant. Within 15–75 min of sampling, the blood samples were centrifuged for 40 min at 4 °C and the plasma stored at −80 °C until assayed.

2.3. Leptin plasma assay

Total leptin levels in human plasma were measured using a commercially available radioimmunoassay (RIA) kit (Linco Human Leptin RIA kit; Linco Research, St. Charles, MO, USA). The antibody in this kit was raised against a highly purified human leptin.

2.4. Leptin kinetics

Renal leptin clearance was calculated as the product of the fractional extraction of plasma leptin in transit through the