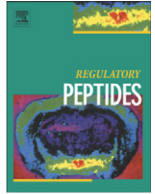
Regulatory Peptides 184 (2013) 75–80

Contents lists available at SciVerse ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep



Lateral hypothalamic serotonin is not stimulated during central leptin hypophagia

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ARTICLE INFO

Article history:
Received 2 February 2012
Received in revised form 12 September 2012
Accepted 3 March 2013
Available online 19 March 2013

Keywords:
Adipokines
Food intake
In vivo microdialysis
Leptin
Serotonin

ABSTRACT

Whether leptin targets the hypothalamic serotonergic system to inhibit food intake is not established. We examined the effect of a short-term i.c.v. leptin treatment on serotonin microdialysate levels in rat lateral hypothalamus. Adipose tissue gene expression was also evaluated.
Male rats received four daily injections of leptin (5 μg) or vehicle (with pair-feeding to leptin-induced intake) and a fifth injection during collection of LH microdialysates. We found that serotonin and 5-HIAA levels were not affected by the leptin pre-treatment, as basal levels were similar between the leptin and the pair-fed group. These levels remained unaltered after the acute leptin injection.
For gene expression studies, rats were pre-treated with five daily injections of either leptin (5 μg) or vehicle (with either pair-feeding or ad libitum intake). mRNA levels of resistin, adiponectin, lipoprotein lipase, and PPAR-gamma were unaltered by either leptin or pair-feeding. Leptin gene expression was significantly reduced by leptin but not by pair-feeding, in both the retroperitoneal (−74%) and the epididymal (−99%) depots while no differences were observed in the subcutaneous depot.
The observations confirmed the absence of an acute stimulatory effect of central leptin on serotonin release in the lateral hypothalamus and showed that the pre-treatment with leptin failed to modify this pattern. This indicates that components of the serotonergic system are probably not directly affected by leptin. Additionally, the central effect of leptin was able to downregulate its own adipose tissue gene expression in a depot-specific manner while other adipokine genes were not affected.
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1. Introduction

It is well recognized that the adipocyte hormone leptin plays a pivotal role in signaling to the central nervous system (CNS) information about the amount of energy stored in white adipose tissue (WAT), thus influencing the central mechanisms involved in the maintenance of energy balance. This effect results, at least in part, from the stimulatory effect of leptin on central anorexigenic factors such as proopiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), and neurotensin, with a concomitant inhibitory effect on central orexigenic factors such as neuropeptide Y (NPY), agouti related peptide (AgRP), melanin-concentrating hormone (MCH) and orexins [1,2].

The neurotransmitter serotonin has catabolic effects on energy homeostasis, reducing food intake and body weight and stimulating energy expenditure [3–6]. Several lines of studies have examined a putative role of serotonin as one catabolic system targeted by leptin, thus contributing to leptin hypophagia. The results are so far highly inconclusive.

Serotonergic raphe neurons have been shown to lack the long isoform of leptin receptors (Ob-Rb) [7] and serotonin turnover was unaffected in several brain areas of normal rats after 3 days of intraperitoneal leptin infusion [8]. Basal and potassium-stimulated serotonin overflow were unaltered by the addition of leptin to synaptosomes or superfusion preparations of rat hypothalami [9,10]. These findings indicated that leptin did not induce serotonergic stimulation.

However, opposite results have been reported. Serotonin raphe neurons have been found to express Ob-Rb [11–14], peripheral leptin has been shown to activate Ob-Rb signal transduction in brainstem and lateral hypothalamic sites [15,16], and leptin has been shown to stimulate hypothalamic serotonin turnover [17,18]. These data favor a stimulatory role of leptin on the serotonergic system.

Functional studies have also led to contradictory findings, as leptin hypophagia has been found to involve activation of 5-HT2B and 5-HT2C receptors [19,20] while it reportedly failed to be affected after serotonergic depletion or 5-HT2C receptor knockout [21].

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0167-0115/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.regpep.2013.03.027

We have previously demonstrated that one single central leptin injection failed to "per se" alter serotonin extracellular levels in the lateral hypothalamus (LH) while it exacerbated the feeding-induced serotonin levels. These findings suggested that, rather than interacting directly with components of the serotonergic system, leptin probably targeted the mechanisms involved in the serotonergic-stimulating effect of food [22].

In view of the controversial findings mentioned above, we performed the present study with the objective of investigating further the ability of leptin to interact with the serotonergic system. To specifically evaluate whether a series of repeated leptin central injections would evidence an effect independent of food intake, we measured extracellular levels of 5-HT and its metabolite 5-hidroxyindolacetic acid, in the lateral hypothalamus of rats pre-treated with leptin or vehicle for 4 days. We chose the in vivo brain microdialysis technique, which allows a direct measurement of 5-HT extracellular levels in the awake animal.

Besides the central effect of leptin on neurotransmitters and neuropeptides involved in energy balance, it has been postulated that central leptin also modulates WAT activity. A recent study demonstrated that a 7-day central leptin infusion up-regulated WAT mRNA and protein levels of resistin but failed to modify resistin plasma levels [23]. In addition, it has been demonstrated that leptin inhibited its own expression in adipocytes via beta 3 adrenoceptor-mediated sympathetic activity [24]. These findings agree with other studies showing that the central effect of leptin on WAT activity or metabolism occurred via the sympathetic nervous system [25–27]. It is possible that central leptin modulates WAT gene expression of other adipokines involved in energy metabolism.

Additional experiments were then performed to evaluate the effect of the leptin treatment on leptin, adiponectin, resistin, PPARγ and lipoprotein lipase gene expressions, in epididymal, retroperitoneal and subcutaneous depots of white adipose tissue.

2. Materials and methods

2.1. Animals and surgery

The Committee on Animal Research Ethics of the Federal University of São Paulo approved the procedures used in the present experiments. Since weaning, male Wistar rats were housed five per cage and maintained in controlled conditions of lighting (12 h light: 12 h dark) and temperature (24 ± 1 °C), with free access to standard balanced rat chow (4% fat, 22% protein, Nuvital Nutrients, Columbo, PR, Brazil) and water.

At 12 to 14 weeks of age the animals used for microdialysis experiments were anesthetized with ketamine/xylaxine (67/13 mg/kg) and stereotaxically [28] implanted with both a 21-gauge guide cannula aimed at the right lateral hypothalamus (A −2.5 mm, L −1.6 mm, V −7.7 mm from bregma) and a 23-gauge cannula aimed at the left lateral ventricle (A −0.9 mm, L +1.6 mm, V −2.6 mm from bregma). For the animals used for northern blotting experiments, a single brain cannula was implanted, aimed at the left lateral ventricle. The cannulas were secured to the skull with screws and dental cement and the animals were individually caged thereafter.

2.2. Microdialysis experiments

Four days after surgery, the animals were divided in 2 groups. The leptin group received 4 daily i.c.v. injections (5 μL) of 5.0 μg of leptin and was fed ad libitum. The pair-fed control group consisted of animals receiving 4 daily i.c.v. injections of vehicle (CSF, artificial cerebrospinal fluid) and being pair-fed to the leptin animals. Food intake was recorded 24 h after each injection and body weight was recorded 24 h after the first 3 injections. The injections were performed around noon.

At 18:00 h, after the fourth injection, a concentric custom-constructed microdialysis probe (1.5 mm of effective membrane length) was inserted through the hypothalamic cannula and fixed to it with a small drop of dental cement. The details of probe construction have been previously described [29,30]. The animals were then connected to a swivel system, which allowed continuous probe perfusion with CSF by a microperfusion pump (Carnegie Medicin, Solna, Sweden). CSF composition was: 145 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 2.0 mM Na_2HPO_4, pH 7.4. Overnight perfusion was performed at 1.0 μL/min.

At 6:00 h, probe flow rate was adjusted to 2.5 μL/min and food was withdrawn from the cage, so that the animals were fasted for 6 h before the microdialysis experiments. Collection of 20-min dialysate samples was started around noon. Samples were collected into 10 μL of 0.5 M perchloric acid and immediately injected into an HPLC system. Baseline samples were collected until 5-HT levels were stable, the last three samples being averaged to yield the mean baseline level (100% value). 5 μL of CSF vehicle was then injected through the i.c.v. cannula and three additional samples were collected (samples V1–V3). An i.c.v. injection of 5 μg of leptin was then administered to all animals. Nine additional 20-min dialysate samples were collected (samples L1–L9). No food was provided during microdialysate sampling.

2.3. HPLC analysis

Dialysate levels of 5-HT and 5-HIAA were measured by high performance liquid chromatography with electrochemical detection. The system (ESA Inc., Chelmsford, Mass.) consisted of a model 580 pump with two PEEK pulse dampers in series, a 50 μL Rheodyne PEEK sample loop, a 3 μm MD150 C column, a model 5020 guard cell set at 300 mV, a model 5014B analytical cell set at −175 and 150 mV, and a model 5200A detector. The mobile phase consisted of 75 mM sodium phosphate, 1.5 mM octanesulfonic acid, 50 μM EDTA, 100 μL/L triethylamine, and 10% v/v acetonitrile at pH 3.0. The flow rate was 0.6 mL/min. The detection limit for 5-HT was 1.5 pg/50 μL at a signal to noise ratio of 3:1.

2.4. Histological analysis

For verification of microdialysis membrane and i.c.v. cannula positioning, at the termination of the experiments all animals were deeply anesthetized and perfused with 0.9% saline followed by 10% formalin and 40 μm brain sections were examined under a microscope, following staining with Cresyl Violet. Only data from rats with correct probe and cannula placements were included in the analysis.

2.5. Northern blotting analysis

Three groups were performed for northern blot experiments. One group received 6 daily i.c.v. injections (5 μL) of 5.0 μg of leptin and was fed ad libitum (leptin group). Two control groups were performed, consisting of animals receiving 6 daily i.c.v. injections of vehicle (CSF, artificial cerebrospinal fluid) and either fed ad libitum (Ad libitum group) or pair-fed to the leptin rats (pair-fed group). The injections were performed around noon. After the fifth injection, at 6:00 a.m., food was withdrawn and, at noon, the sixth injection was administered. One hour later, the animals were anesthetized, killed by decapitation, and samples from epididymal, retroperitoneal and subcutaneous white adipose tissue depots were quickly removed and inserted into vials containing RNA later® (Ambion) and frozen at −70 °C. RNA extraction was performed according to the method described by [31]. Adipokines mRNA levels were measured by Northern blotting using antisense oligonucleotide probes in conjunction with chemiluminescence detection [32]. Signals were collected by exposure of the membrane to X-ray film and autoradiographs were quantitated by densitometry with image-analysis software (MCID Basic Software—Image Research Inc.).

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