Peptides 56 (2014) 59–67

Contents lists available at ScienceDirect

Peptides

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

Age versus nutritional state in the development of central leptin resistance

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ARTICLE INFO

Article history:
Received 29 October 2013
Received in revised form 5 March 2014
Accepted 9 March 2014
Available online 25 March 2014

Keywords:
Leptin
Aging
Obesity
Calorie-restriction
Energy balance

ABSTRACT

Leptin, a catabolic adiposity signal acts in the hypothalamus via suppressing food intake and inducing hypermetabolism. Age and obesity are accompanied by leptin resistance. The present study aimed to clarify which components of the catabolic leptin effects are influenced most strongly by aging and which ones by nutritional state-induced alterations in body composition. In our biotelemetric study the effects of a 7-day intracerebroventricular leptin infusion on various parameters of energy balance (food intake, body weight, oxygen consumption, heart rate and body temperature) were analyzed in male Wistar rats of different age-groups (from 3 to 24 months) and nutritional states (normally fed, diet-induced obese and calorie-restricted). Leptin resistance of older animals affected hypermetabolic actions, whereas leptin induced anorexia in all age-groups. Weight reducing effect of leptin diminished in middle-aged and aging animals to become significant again in the oldest group. In diet-induced obese rats leptin-induced hypermetabolism of the young rats and hypermetabolism plus anorexia of the aging ones were suppressed. Calorie-restriction reduced body weight and fat mass to a similar extent in all age-groups. It strongly enhanced leptin-induced hypermetabolism at all ages and prevented the manifestation of anorexigenic actions of leptin with the exception of the oldest group. This latter finding suggests an unexpected increase of responsiveness to anorexigenic leptin actions in old rats. Accordingly, anorexia and hypermetabolism change in disparate ways with aging. Nutritional state predominantly influences hypermetabolic leptin actions. Resistance to both hypermetabolic and anorexigenic actions were promoted by obesity, while calorie-restriction enhanced responsiveness to leptin, especially in old rats.

1. Introduction

Leptin is known as a peptide produced mainly, though not exclusively, in the white adipose tissue, proportionally with the amount of fat mass [12]. It is regarded as probably the most important peripheral feedback signal to hypothalamic nuclei involved in the long-term central regulation of energy balance [5]. The peptide passes through the blood–brain barrier via a special transport system [44] and binds to its receptors. Activation of its receptors in the arcuate nucleus on the one hand activates anorexigenic–catabolic mechanisms conveyed by pro-opiomelanocortin (POMC) derived melanocortins (primarily melanocyte stimulating hormone, MSH) and cocaine–amphetamine regulated transcript (CART), while on the other hand it inhibits orexigenic–anabolic pathways connected with neuropeptide Y (NPY) and perhaps also with agouti-related peptide (AgRP). Peptides of the second order neurons (orexins, corticotropin releasing factor (CRF), etc.) downstream to the cells of the arcuate nucleus contribute to these mechanisms [38,39]. Increasing leptin levels are assumed to prevent overeating and to enhance energy expenditure in order to prevent further energy (fat) accumulation, while the effects are the opposite in case of decreased leptin levels (e.g. suppression of energy loss during starvation). Congenital lack of leptin, abnormality of the peptide's or its receptor's structure are all accompanied by severe obesity [12,43].

Abbreviations: AgRP, agouti-related peptide; AMPK, adenosine monophosphate kinase; BPM, beats per minute; BW, body weight; CART, cocaine-amphetamine regulated transcript; CR, calorie-restricted; CRF, corticotropin releasing factor; FI, food intake; HF, high-fat diet-induced obese; HR, heart rate; ICV, intracerebroventricular; IP, intraperitoneal; JAK2, Janus kinase-2; MAPK, mitogen-activated protein kinase; MSH, melanocyte stimulating hormone; NF, normally fed; NPY, neuropeptide Y; PFS, pyrogen-free saline; PI3K, phosphoinositide-3-kinase; POMC, pro-opiomelanocortin; PTP-1B, protein tyrosine phosphatase-1b; SOCS-3, suppressor of cytokine signaling-3; STAT3, signal transduction and activator of transcription-3; Tc, core body temperature.

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http://dx.doi.org/10.1016/j.peptides.2014.03.011
0196-9781/

It has long been recognized that the responsiveness to leptin changes both with adiposity [18] and with age [30]. In particular, suppression of food intake by leptin was demonstrated to decrease with age-related obesity [31]. The question remained whether leptin resistance is due to age per se or rather to the concurrent increase in fat mass. Food intake was found to be strongly suppressed by leptin injection or infusion in young but not in old rats, i.e. with age a certain leptin resistance developed [30]. Resistance developed already at a young age in rats with diet-induced obesity [35]. Calorie-restriction that reduces fat mass may prevent leptin-resistance. However, failure of calorie-restriction to restore leptin responsiveness in aged rats, when tested on food intake alone, has been reported [14]. In contrast, a transient three-month food deprivation in rats was demonstrated to re-establish leptin responsiveness even at later ages, at least regarding suppression of food intake [11]. Unfortunately, other parameters of leptin effects, in particular those related to the metabolic effects like metabolic rate, locomotor activity, core temperature, were not in the focus of previous studies.

The present study aimed to clarify which components of the catabolic leptin effects are influenced most strongly (and/or earlier) by aging and which ones by nutritional state-induced alterations in body composition.

The effects of a 7-day long large dose intracerebroventricular (ICV) leptin infusion on daily food intake, body weight, body temperature, heart rate (representing metabolic rate [6]), and spontaneous activity were investigated in normally fed male Wistar rats aged 3, 6, 12, 18 or 24 months. In the 6-, 12-, 24-month age-groups a number of rats were maintained on a calorie restricted diet from age 2 months. For further comparison a subgroup of 6- and 18-month old rats with diet-induced obesity were also infused ICV with leptin to analyze the effects of the peptide on parameters of energy balance.

2. Methods

2.1. Animals, assessment of food intake and growth rate

Male Wistar rats from the colony of the Department of Pathophysiology and Gerontology of the University of Pecs, Hungary were used in the experiments. After weaning the animals were kept individually in plastic cages (375 mm × 215 mm, height 149 mm, covered by steel grid, equipped with feeder and bottle container) with some wood-chip bedding at an ambient temperature of 22–25 °C. Although long-term isolation may alter the behavior of the animals, in order to measure the daily food intake and to provide the allotted portion of food in the calorie-restricted groups, it was necessary to use individual caging. However, the animals were handled on a daily basis. The lights were on between 06.00 and 18.00 h. Powdered standard rat chow (CRLT/N rodent chow, Szindbad Kft., Godollo, Hungary, 11 kJ/g) and tap water were continuously available. The pellets had to be powdered in order to avoid hoarding behavior to allow the measurement of daily food intake. A subgroup of the animals was calorie-restricted from age 2 months onwards: they received 2/3rd of the normal daily amount of standard powdered chow (16 g/day), with vitamin and mineral supplementation and unlimited water intake. Another high-fat diet-induced obese subgroup was established using Diet Induced Obesity Rodent Purified Diet with 60% Energy from Fat, IPS TestDiet (21.6 kJ/g) from age 2 months. The powdered version of the high-fat diet contained 10% normal powdered chow admixed to the powdered high-fat pellets (20.54 kJ/g). The following groups were tested in the experiments: (1) normally fed (NF) animals at ages 3, 6, 12, 18 and 24 months (NF3, NF6, NF12, NF18 and NF24, representing human young adult, younger and older middle-aged, aging and old populations, respectively); (2) calorie-restricted (CR) animals of three age-groups (CR6, CR12 and CR24); (3) high-fat diet-induced obese 6- and 18-month old rats (HF6 and HF18 – the HF rats usually died before the age of 24 months).

Body weight (BW) and spontaneous daily food intake (FI) were measured every day at 09.00 h – thereby the animals were also accustomed to regular handling. The responsiveness to ICV leptin infusion on the regulation of energy balance was checked in all groups in a biotelemetric system (MiniMitter VMFH series 4000, Sunriver, OR).

2.2. Surgeries, drug administration

Transmitter implantation was performed according to our previous studies [26,35] under intraperitoneal (IP) ketamine + xylazine [78 mg/kg (CALYPSOL, Richter) + 13 mg/kg (SEDAXYLAN, Eurovet)] anesthesia right before placing the animals into the biotelemetric MiniMitter system. After one week of adaptation they had a second operation under similar anesthesia (day 0, between 09.00 and 15.00 h), this time an implantation of an ICV cannula (Alzet Brain Kit) into the right lateral cerebral ventricle as we have described earlier in details [26]. At the same time an Alzet osmotic minipump filled with leptin (recombinant leptin, Bachem) or pyrogen-free saline (PFS) was inserted underneath the skin of the nape which was connected to the outer end of the brain kit. The infusion reached the brain after 8–10 h and secured a standard slow ICV infusion (1 μg/μl/h leptin or 1 μl/h PFS) for a period of 7 days. The anesthesia and the surgery severely influenced all parameters, therefore values of day 0 (except for BW measured on the morning preceding the operation) were excluded from all analyses.

2.3. Studies on energy expenditure

Parameters reflecting energy expenditure were analyzed in our biotelemetric system. Animals from each group had a transmitter implanted IP, then they were placed into the MiniMitter cage. The transmitters conveyed signals of core body temperature (Tc), heart rate (HR, for indirect assessment of metabolic rate, [6]) and spontaneous (horizontal) locomotor activity of freely moving animals. The receiver was placed underneath the MiniMitter chamber. The automatically recorded 5-min data were collected and integrated into two mean values per day, one characterizing values of the daytime (resting) period (photophase), another one the nighttime (active) period (scotophase). For data analysis the VitalView software provided by the manufacturer was used [15]. By the help of an attached food container the daily food intake was manually measured every day, together with body weight.

To test the validity of heart rate monitoring for assessment of metabolic rate, measurements of oxygen consumption were performed by indirect calorimetry (Oxymax, Columbus, OH). Freely moving rats from NF12 and CR12 groups were placed singly into ventilated 30 cm × 25 cm × 20 cm plexiglass metabolic chambers at an ambient temperature of 25 °C, with food (according to their feeding schedule) and water available ad libitum. From the air perfusing the chamber the O_2-consumption, CO_2-production were measured in 10-min intervals for a few days prior to and throughout the 7-day PFS or leptin infusion period (except for a 20-min period/day when the chamber was opened for cleaning at 09.00 h). The results were collected on a computer and the data were averaged for day- and nighttime 12-h periods according to the circadian rhythm.

2.4. Post mortem examinations

After the ICV infusion (day 8) the rats were euthanized by an overdose of urethane. Indicators of body composition of NF, CR and HF rats were determined: the wet weights of the anterior