



Ageing increases SOCS-3 expression in rat hypothalamus: effects of food restriction

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Abstract

Aged Wistar rats are characterized by leptin and insulin resistance. The expression of SOCS-3 in hypothalamus increases with ageing. Food restriction during 3 months decreases obesity Lee index in aged rats with respect to their ad libitum aged-mates and brings serum leptin concentrations to values close to those of young rats. Food restriction partially reverts the increases in SOCS-3 mRNA levels associated with ageing. These results suggest that SOCS-3 may be a mediator of hypothalamic leptin resistance in the aged Wistar rat and that the hyperleptinemia associated with ageing is, at least in part, responsible for the increase of SOCS-3 expression in hypothalamus. © 2002 Elsevier Science (USA). All rights reserved.

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Leptin, the product of the *ob* gene, is mainly produced and secreted by the adipose tissue. It enters the brain from circulation where it plays a key role in the regulation of neuroendocrine axis and energy balance, leading to the reduction of food intake and energy expenditure [1,2]. The action of leptin is mediated by the leptin receptor (Ob-Rb), a splicing variant characterized by a long cytoplasmic C-tail, belonging to the cytokine receptor superfamily [3]. Upon binding to its receptor, leptin activates a cytokine-like signal transduction pathway, involving the stimulation of JAK-STAT cascade [4]. Impairment of any of the above-mentioned molecular events may result in deficient leptin signaling and hyperleptinemia, leading to a state of leptin resistance. Obesity and ageing are circumstances associated with hyperleptinemia and leptin resistance in different rodent models [5–7]. Besides a possible impairment at the level of blood–brain barrier [7], alterations in the signal transduction pathway at central level, like atten-

uation of STAT-3 phosphorylation or decrease in the expression of leptin receptor have also been described in aged Fisher [8,9] and Wistar rats [6,7,10].

As other members of the cytokine superfamily such as growth hormone [11], leptin increases SOCS-3 mRNA levels in hypothalamus [12]. SOCS-3 is a member of the family of the suppressor-of-cytokine-signaling, which inhibits JAK activity, suggesting a role for this protein in a negative feed-back loop, which modulates leptin signaling. The fact that SOCS-3 mRNA levels are elevated in arcuate and dorsomedial hypothalamic nuclei of A^y/a mice, a murine model of leptin resistance has led to the suggestion that SOCS-3 may be a mediator of leptin resistance [12]. In the present article, we have explored the expression of SOCS-3 in the hypothalamus of the aged Wistar rat, a model characterized by insulin and central leptin resistance [13,10]. The fact that central leptin resistance is attenuated in this model by long-term food restriction suggests that the increase of adiposity associated with ageing plays a key role in the development of central leptin resistance [10]. Therefore, the expression of SOCS-3 has also been investigated in food restricted aged rats.

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Materials and methods

Animals. Wistar albino male rats aged 3, 7, and 23 months were used throughout the study. The animals were obtained from the “Centro de Biología Molecular Severo Ochoa” (Madrid, Spain). Rats were housed individually in climate-controlled quarters with 12 h light cycle and handled following the European Union laws and NIH guidelines. Animals were fed standard laboratory chow. Special care was taken to minimize animal suffering and to reduce the number of animals used.

Food restriction. Four- and 20-month-old rats were placed in individual cages and fed daily with an amount of chow equivalent to 75–80% of the normal food intake [10], until they reached a body weight equivalent to 75% of that of ad libitum fed aged-mates. Usually, this was achieved 1 month from the start of nutritional restriction. Animals were weighed weekly and the amount of food provided was adjusted individually to maintain the weight. Animals were used at the ages of 7 and 23 months, respectively.

RNA extraction and RT-PCR. Hypothalamus was dissected and total RNA was isolated using RN-Easy Mini Kit (Qiagen-Ref. 74104). The cDNA was synthesized from 5 µg DNase-treated RNA [14] by using the reverse-transcriptase activity from Moloney murine leukemia virus (Gibco-BRL) and pd[N]₆ (Boehringer) as random primer. The reactions were performed in 20 µl and then diluted to 100 µl, of which 15 µl was used as a template for SOCS-3 PCRs and 5 µl for actin ones. The forward primer for SOCS-3 PCR was 5'-ACCAGCGCCACTTCTTACA-3' and the reverse primer was 5'-GTGGAGCATCATACTGGTCC-3'. Both were synthesized according to the published sequence for SOCS-3 [15] and flank a 450 bp fragment. The rat actin primers were 5'-GGTATGGAATCCTGTGGCATCCATGAAA-3' for the 5' end and 5'-GTGTA AACCGCAGCTCAGTAACAGTCC-3' for the 3' end. Both primers flank a 356 bp fragment [16]. The PCRs were performed in a total volume of 50 µl with 1 U thermostable DNA polymerase from *Thermus thermophilus* (Biotools). The template was denatured for 5 min at 94 °C, followed by 35 cycles (30 cycles for actin amplification) with the following temperatures: denaturation 1 min at 94 °C, annealing 2 min at 55 °C, and elongation for 1 min at 72 °C. Reactions were finished with a final extension of 10 min at 72 °C. After reaction, the samples were electrophoresed in 2.5% agarose gels and the amplified bands were analyzed with Kodak digital science 1D program. Relative amount of amplification was calculated in each sample and expressed as ng amplified cDNA from SOCS-3/ng amplified cDNA from actin used as control for amplification. Control reactions with different number of cycles had been performed previously to select conditions of amplification in the linearity range.

Other methods. Obesity Lee index was calculated, as indicated in [5]. Blood glucose concentration was determined using a glucose analyzer (Accutrend, Roche). Serum leptin concentration was measured using a Radioimmunoassay Kit (Linco).

Statistical analysis. Statistical comparisons were performed by Student's *t* test.

Results and discussion

In agreement with previous results [6], Table 1 shows that body weight increases in Wistar rats with ageing without significant changes in obesity Lee index. Aged rats remain normoglycemic but exhibit a progressive and marked increase in plasma leptin concentrations. Food restriction during 3 months keeps body weight to about 75–80% of ad libitum fed aged-mates and elicits a significant decrease of obesity Lee index in 7- and 23-month-old rats. Additionally, food restriction brings plasma leptin concentrations in 7-month-old rats to values below those of young rats and also decreases leptin levels in 23-month-old animals to values similar to those of 7-month-old rats fed ad libitum (Table 1). The data of Fig. 1 clearly show that hypothalamic SOCS-3 mRNA levels increase significantly in the Wistar rat with ageing. Similar results have been previously reported by Wang et al. [17] in lean wild-type ZDF old rats. The increment in the expression of SOCS-3 in aged rats correlates with the described decreased responsiveness to centrally administered leptin of aged Wistar rats [10]. This agrees with the data of Bjorbaek et al. [12] who described an increase in the levels of SOCS-3 mRNA in another rodent model of leptin resistance such as the murine A^y/a, suggesting that SOCS-3 may be a mediator of leptin resistance.

As old rats present an increase in adiposity and subsequent hyperleptinemia, any of the effects observed in aged rats might be due to ageing-associated adiposity, ageing by itself or both. Therefore, in an attempt to discriminate between the effects of ageing-associated adiposity from those of ageing, 4- and 20-month-old rats were maintained under food restriction for 3 months. Food restriction decreases SOCS-3 expression in the hypothalamus (Fig. 1) in parallel with the decrease of plasma leptin concentrations observed in both groups of aged food restricted rats (Table 1). These data suggest that hyperleptinemia associated with ageing is, at least

Table 1
Characteristics of the rats

	3 months	7 months	7 months-FR	23 months	23 months-FR
Body weight (g)	438 ± 17	562 ± 21 ^b	422 ± 9 ^a	778 ± 22 ^{b,c}	607 ± 13 ^a
Blood glucose (mg/dl)	123 ± 4	131 ± 5	109 ± 4 ^d	126 ± 4	125 ± 4
Serum leptin (ng/ml)	4.6 ± 0.5	7.5 ± 0.9 ^c	2.0 ± 0.1 ^a	27.6 ± 4.0 ^{b,c}	7.1 ± 1.2 ^a
Lee index	311 ± 3	315 ± 4	297 ± 3 ^d	319 ± 3	302 ± 4 ^d

Data are means ± SEM of 10–12 animals per group.

^a *p* < 0.001 versus same age fed ad libitum.

^b *p* < 0.001 versus 3 months.

^c *p* < 0.001 versus 7 months.

^d *p* < 0.01 versus same age fed ad libitum.

^e *p* < 0.05 versus 3 months.

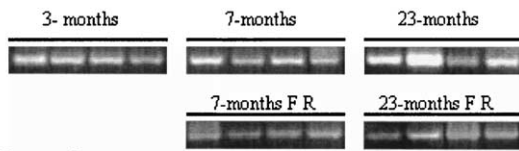
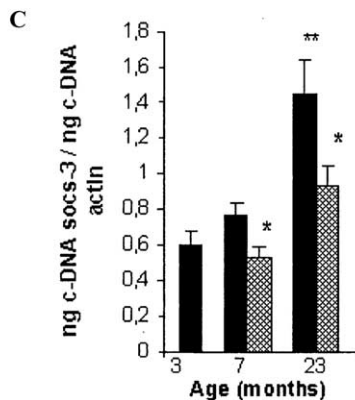
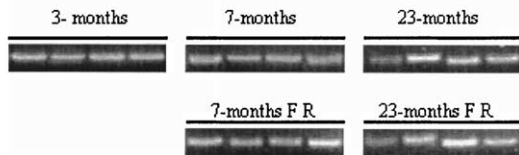
A Socs-3 Expression**B Actin Expression**

Fig. 1. Changes in hypothalamic SOCS-3-expression with age and food restriction. Expression of SOCS-3 in hypothalamus from 3-, 7- and 23-month old rats as well as their nutritionally restricted aged-mates (FR) was determined by RT-PCR as indicated in Materials and methods and quantified as the ratio of ng of SOCS-3 cDNA amplified per ng cDNA of actin under the reaction conditions used. (A) cDNA amplified from SOCS-3. (B) cDNA amplified from actin. (C) Quantification analysis. (■) Fed ad libitum and (▨) food restricted. Values are means \pm SEM of five separate determinations per group of animals. ** $p < 0.01$ versus other ages, * $p < 0.05$ versus same age fed ad libitum.

in part, responsible for the increase of SOCS-3 expression in hypothalamus. In fact, Wang et al. [17] have recently reported that after adenovirus-induced hyperleptinemia in young lean wild-type ZDF rats, SOCS-3 mRNA expression in the hypothalamus increased to the level of the old ZDF rats. This is in agreement with the above-mentioned idea that, at least, part of the central leptin resistance is due to the increase of adiposity associated with ageing. Nevertheless, while in 7-month-old rats food restriction brings the SOCS-3 expression to levels similar of those of young animals, in food restricted 23-month-old rats the levels of SOCS-3 mRNA are still higher than those of young ones, suggesting that ageing by itself also plays a role in the development of leptin resistance. In this sense, Ilan et al. [18] have demonstrated that aged Sprague–Dawley rats present resistance to peripherally administered leptin in spite of being under food restriction since postpuberty. Other

alterations at central level like impairment in the signal transduction pathway at the level of STAT-3 phosphorylation or decrease in the expression of leptin receptor, have also been described in aged Fisher [8,9] and Wistar [6,10] rats. More work would be needed to clarify whether or not these alterations are mediated by SOCS-3 and the relative effect of ageing by itself versus that of the ageing-associated adiposity.

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