Effects of Growth Hormone on Leptin Gene Expression in Rats

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RECENTLY the obesity gene was identified and its gene product, leptin, has demonstrated [1]. It is expressed exclusively in adipocytes, secreted into the blood stream and appears to signal to the hypothalamus through its specific receptor. It reduces appetite and stimulates energy expenditure. Thus it serves an endocrine function in body weight regulation of energy consumption and storage. Since leptin plays a pivotal role in the regulation of adiposity and energy homeostasis, the level of its expression is likely to fluctuate under various physiological, nutritional and disease conditions.

On the other hand, GH-deficient patients are often associated with increase body fat and GH treatment in GH-deficient patients significantly reduced the total fat mass and intra-abdominal adipose tissue [2]. Some reports have demonstrated the beneficial effect of GH administration in obese [3–5] or obese diet-restricted humans [6]. We therefore studied the effect of GH on leptin gene expression in order to identify the possible role of leptin in GH treatment for obese subjects.

Materials and Methods

Male Wistar rats hypophysectomized or sham operated at the 5th week were obtained from Nihon Animals Inc. (Osaka, Japan). Male Zucker (fa/fa) rats and their lean littermates (Fa/?) were obtained from Kiwa Laboratory Animals Co., Ltd. (Wakayama, Japan). Recombinant human (rh) GH was obtained from Pharmacia & Upjohn, Stockholm, Sweden and was dissolved in H2O and injected s.c. once a day for 7 days (1.5 IU/kg). Saline was injected to the control rats. On the day of sacrifice, the animals were anesthetized and epididymal, omental and subcutaneous fat tissues were excised and immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction.

Total RNA was extracted by the guanidine/CsCl method. Leptin mRNA levels were determined by reverse transcription-polymerase chain reaction (RT-PCR) according to the method previously reported [7]. RNA samples were reverse transcribed with oligo (dT)15 or the reverse primer for the PCR. The primers for PCR were selected with a computer program, Primer 3, White Head Institute for Biomedical Research, MIT, Cambridge, MA. The rat leptin primers were from a sequence with accession number D49653 [8]. The forward primer (118 to 138 nt) was 5'-ACACCAAAA CCCTCATCAAGA-3' and the reverse primer (301 to 283 nt) was 5'-GAAGGCAAGCTGGTGAGGA-3'. The expected size of the PCR product was 184 bp. PCR was carried out as previously reported [7]. The annealing temperature was 65°C and the number of PCR cycles was 40 for quantitative PCR for leptin mRNA. The PCR products were run on 3% agarose gels and stained with ethidium bromide. After taking photographs, the amount of PCR product was determined with a computer program, Image, NIH, Bethesda, MD. Statistical analysis was performed by Student's t-test.
Results and Discussion

In order to clarify the mechanism of the obesity in patients with hypopituitarism, we first investigated leptin mRNA in hypopituitary (hypox) rats. The body weight of the hypox rats was about half that of the control rats which were sham operated (133.5 ± 2.52 g vs. 253.67 ± 2.08 g). As shown in Fig. 1, the leptin /GAPDH mRNA ratio in epididymal fat pad of hypox rats was significantly lower than in control rats. These data are compatible with the recent report of Bonis-Schnetzler [9], but they suggested the amount of leptin mRNA in the fat tissue is dependent of the body weight. For this reason, the lower leptin mRNA levels might be related to the lower body weight of hypox rats but not to the lower levels of GH. Indeed dietary obesity could be induced in the GH-deficient dwarf rat, but no such abnormality in fat deposition as seen in patients with hypopituitarism can be induced in hypox rats [10], so that something other than GH must participate in the abnormal fat deposition in patients with hypopituitarism. Glucocorticoid is one candidate. The administration of glucocorticoid induces ob gene expression with a reduction in body weight and food intake in rats [11], so that glucocorticoid deficiency may decrease ob gene expression in hypox rats. This hypothesis is also supported by a previous report that the administration of GH does not restore the reduced levels of leptin mRNA in hypox rats [8]. The hypox rat is therefore too complicated for the study of GH action on leptin gene expression and obesity.

Recently, the molecular defect in obese Zucker rats has been shown to be similar to that in db/db mice and to involve the receptor for leptin [12]. Obese Zucker fatty rats are resistant to leptin treatment, which is similar to most obese humans. As for GH, the synthesis and secretion of GH in the animal decrease after the onset of obesity as seen in obese children [13]. The decrease in GH secretion is not a primary event but may play a role in the persistence of the obesity, so that Zucker rats may be more suitable for the study of the GH action on obesity. We therefore investigated the effect of GH on leptin mRNA expression in adipose tissue by using the rats. At first we measured leptin mRNA levels in epididymal fat tissues. As shown in Fig. 2, leptin mRNA level in fa/fa rats is higher than that in their lean littermates as expected. In order to investigate the possible beneficial effect of GH on the obesity, we

![Fig. 1. Effects of hypopituitary on leptin mRNA levels in epididymal fat tissues. Quantitative RT-PCR was conducted as described in Materials and Methods. The amounts of PCR products were determined with a densitometer and the results were expressed as the leptin/GAPDH mRNA ratio in arbitrary units. As shown the leptin/GAPDH mRNA ratio of hypox rats was significantly lower than that of sham operated control rats.](https://example.com/fig1.png)

![Fig. 2. Effect of GH on leptin mRNA levels in epididymal fat tissues of Zucker rats. As shown in the figure, the leptin/GAPDH mRNA ratio in control fa/fa rats was higher than that in lean littermates (Fa/?). The high mRNA levels in epididymal fat tissues were lowered by GH administration (rhGH fa/fa) but not in subcutaneous tissues (data not shown).](https://example.com/fig2.png)
administered rhGH (1.5 IU/Kg) for 7 days. We could not demonstrate any differences in body weight or % total fat in these animals (data not shown). As shown in Fig. 2, the leptin mRNA levels in epididymal fat tissue were significantly reduced by GH administration, but we could not demonstrate any significant changes in leptin mRNA in subcutaneous fat tissues (data not shown), so that the effect of GH may be specific for visceral fat tissues. The different metabolic consequences and hormonal responsiveness of visceral vs. peripheral fat tissue accumulation are reported in human adults as well as in Zucker fatty rats [14] and visceral fat tissue is considered to be more important for the etiology for various diseases.

A reduction in leptin gene expression in Zucker fatty rats was reported for chronic administration of an antidiabetic thiazolidinedione agent [15]. The agent improves the resistance to insulin which is usually observed in obese humans. According to the report the reduction in leptin mRNA was accompanied by an increase in body weight gain without an increase in food intake in the obese animals, so that reduction in leptin gene expression might be a consequence of improved metabolism. We have no idea of the exact mechanism of the reduction in leptin gene expression by GH so far, but modification of ob gene expression with these pharmaceutical agents may be a feasible approach to treating obesity or improving the abnormalities in lipid or glucose metabolism in such patients.

References