Growth Hormone (GH) Receptor and IGF-I Receptor Messenger RNA Expression in Peripheral Lymphocytes from Patients with GH Deficiency and Acromegaly

IZUMI FUKUDA, NAOMI HIZUKA, KAZUE TAKANO, TOMOKO KAZAMA, YUMIKO OKUBO, KUMIKO ASAKAWA-YASUMOTO, AND HIROSHI DEMURA

Department of Medicine II, Tokyo Women’s Medical College, Tokyo 162-8666, Japan

GH acts on not only skeletal growth, but also various metabolisms such as anabolism, lipid and electrolytes, and the immune system. The actions of GH are mediated through binding to specific receptors located on cell membranes. The specific receptors for GH have been identified in various tissues such as hepatocytes, cartilage and adipocytes [1-3]. Circulating lymphoid cells as well as lymphoid-derived cell lines also express both GH receptors (GHR) and insulin like growth factor (IGF)-I receptors [4, 5]. Clinical studies with peripheral lymphocytes from relatively small blood collections are useful for evaluating GHR status as tissue biopsies are not required. Studies on the binding of GH to human peripheral lymphocytes have been carried out but little is known about the regulation of GHR in the lymphocytes because there is little binding. The recent development of RT-PCR makes it be possible to measure the expression of mRNA with a small amount of RNA. Therefore, in the present study, we measured GHR mRNA levels in the lymphocytes from patients with GH deficiency (GHD) and acromegaly by using quantitative RT-PCR, and investigated whether the circulating GH and/or IGF-I affects the levels of GHR mRNA. We also measured the receptor mRNA levels of IGF-I, that mediates many effects of GH in endocrine, paracrine and autocrine fashion.

Materials and Methods

Subjects

14 normal subjects (six men and eight women), ten patients with acromegaly (seven men and three women) and ten patients with GHD (seven men and three women) were studied.

Human peripheral lymphocyte preparation

Blood samples (10 ml) were obtained in EDTA tubes from normal adults and patients with acromegaly and GHD. The blood was centrifuged and the plasma was collected for measurement of GH and IGF-I. The blood cells were layered onto Ficoll-paque and centrifuged at 400 × g for 40 min at room temperature. Mononuclear cell fraction was taken as peripheral lymphocytes, and the cells were washed twice with phosphate-buffered saline.

RT-PCR

The PCR primers were synthesized on the basis of the sequence of the human GHR and IGF-I receptor (IGF-IR), and the sequences were as follows. GHR: The sequences in exon 3-4 (5'-CAAATTCTTCTAAGGAGC C-3': sense primer) and exon 5 (5'-TCCACTGTACCACCATTGCTA-3': antisense primer) of hGHR genes. The PCR product was 277 bp. IGF-IR: 5' sense primer specific for codons -32 to -27 of the IGF-IR sequence (5'-AAGGAATGAAGTCTGGCTCC-3') and 3'antisense...
primer complementary to IGF-IR codons 20 to 27 (5'-CTCGATCACCGTGCAGTTCT-3') were used [6]. The PCR product was 173 bp.

Total RNA from peripheral lymphocytes was extracted by the modified acid-guanidine-phenol-chloroform method, and the RNA samples were treated with DNase. The DNase treated RNA was reverse transcribed with Moloney murine leukemia virus transcriptase and oligonucleotide antisense primer. The resulting cDNA fragments were amplified with Taq polymerase and oligonucleotide sense and antisense primers. The 30 cycle and 18 cycle products, which were within the linear logarithmic phase of the amplification curve, were analyzed as GHR (Fig. 1) and IGF-IR genes mRNA, respectively. The generated products were resolved by 3% agarose gel electrophoresis and visualized by ethidium bromide staining. As the internal control, β-actin mRNA levels were measured by RT-PCR simultaneously. The GHR and IGF-IR mRNA levels were adjusted by β-actin mRNA, and expressed as % of the reference sample from a normal subject.

Statistics

The results are given as the mean ± SEM. Student's t-test was used for statistical analysis.

Results

The predicted 277 bp fragment specific for the hGHR cDNA and 173 bp fragment specific for the hIGF-IR cDNA were generated by the RT-PCR in human peripheral lymphocytes, respectively. The GHR mRNA levels in patients with acromegaly and GHD were 63 ± 9, 48 ± 6%, respectively. These levels were significantly lower than those in normal subjects (101 ± 13%) (Fig. 2). The IGF-IR mRNA levels in patients with GHD were significantly higher than those in normal subjects (176 ± 28 vs. 93 ± 17%), but there were no significant differences between patients with acromegaly (93 ± 31%) and normal subjects in IGF-IR mRNA levels (Fig. 3).

Discussion

In the present study, the GHR mRNA levels in patients with acromegaly and GHD were significantly lower than those in normal subjects, and we found no correlations between GHR mRNA levels and serum GH and/or IGF-I levels. The regulation of the GHR by GH was controversial in previous reports. In IM-9 lymphocytes and

---

**Measurements of plasma GH and IGF-I**

Plasma GH was measured with a commercially available IRMA kit (Eiken Chemical Co., Ltd, Tokyo, Japan). Plasma IGF-I levels were measured by means of an RIA kit (Nichols Institute Diagnostics, San Juan Capistrano, CA) with acid-ethanol extracted samples.

**Fig. 1.** Quantitation of GHR mRNA expression in peripheral lymphocytes by RT-PCR (30 cycle products).
fibroblasts, GH has been shown to reduce its receptor [7, 8] but Mathews et al. reported that hypophysectomy and GH treatment did not affect hepatic GH receptor mRNA levels in rats [9]. Maiter et al. observed that continuous infusions of GH increased the number of GHR in liver, although the pulsatile administration of GH did not change the number of GHR in hypophysectomized rats [10]. Taking these findings together, they indicate that GH stimulates the synthesis of its liver receptors as well as their degradation. This might be one mechanism that causes the lack of a correlation between GHR mRNA levels and circulating GH levels, as the net effect of these opposing actions could vary the GHR levels after GH exposure.

In this study, the IGF-IR mRNA levels in patients with GHD were significantly higher than those in normal subjects. Our findings are consistent with previous data that suggest IGF-I decreases IGF-I receptor. This phenomenon was originally described in vitro by using IM-9 lymphocytes and cultured human fibroblasts [11, 12], and has been supported by the report of an inverse correlation between plasma IGF-I levels and receptor concentrations in hypopituitary children treated with GH [13]. The IGF-I binding sites on erythrocytes also increased in patients with a chronic low IGF-I serum concentration [14]. We confirmed these findings by measuring IGF-IR mRNA levels in human peripheral lymphocytes by the RT-PCR method.

Hizuka et al. reported that the decrease in IGF-I receptor in erythrocytes was observed in four acromegalic patients in the binding studies [15]. In contrast with their results, there were no significant differences between patients with acromegaly and normal subjects in IGF-IR mRNA levels in this study. These different results could not be clearly explained. However, one possibility is that IGF-I regulates IGF-I receptor not at mRNA level but at post transcriptional levels.

In conclusion, we studied GHR and IGF-IR mRNA levels in patients with GHD and acromegaly by quantitative RT-PCR. GHR and IGF-IR mRNA levels in peripheral lymphocytes did not correlate with either plasma GH or IGF-I levels. The regulation of GHR and IGF-IR mRNA levels in peripheral lymphocytes by circulating GH and/or IGF-I was not fully understood from these data but the levels might be partly affected by GH and/or IGF-I. Further investigation will be required.
References


