Adrenocorticotropic Hormone (ACTH) Increases the Expression of Its Own Receptor Gene

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Abstract. Regulation of the ACTH receptor (R) in the adrenal gland by its own ligand "ACTH" has been a matter of controversy. In the present study, whether ACTH regulates the expression of mRNA for its own receptor in the adrenal gland was studied in human subjects and in rats in vivo. In the human study, adrenal adenoma tissues as well as adjacent normal tissues were obtained at surgery from two patients with typical Cushing's syndrome. Northern blot analysis revealed two ACTH-R mRNA species with 4.0 kb and 2.0 kb. ACTH-R mRNAs in the adenoma tissues were much more abundant than those in the normal tissues from the two patients, suggesting that the mRNA in normal adrenal tissue is either suppressed by cortisol excess or the absence of ACTH. To examine the mechanism involved in ACTH-R mRNA regulation, the changes in the receptor mRNA caused by ACTH were studied in dexamethasone-treated rats. Administration of dexamethasone for 5 days resulted in a marked decrease in ACTH-R mRNA to an undetectable level. A bolus administration of ACTH1-24 intravenously or ACTH-Z1-24 intramuscularly to the dexamethasone-treated rat did not cause any significant change in ACTH-R mRNA from 0.5 to 12 h after the administration. However, a significant increase in the receptor mRNA was observed at 24 h after the ACTH-Z1-24 and the level was further increased until 48 h followed by a sustained increase at 72 h when it was given once every 24 h. These data suggest that the ACTH-receptor is increased by ACTH at a pretranslational level. Although it remains to be studied whether the increased receptor mRNA level in the adenoma tissues of the patients with Cushing's syndrome is a general phenomenon, the results suggest that the regulatory mechanism of the receptor in the adenoma is different from that in normal tissue and this could contribute to the pathogenesis of autonomous production of cortisol.

Key words: ACTH receptor, mRNA, Rat adrenal, Dexamethasone

REPEATED doses of hormone have the effect of diminishing the response of target endocrine tissues [1]. This desensitization or a refractory response is often associated with a decrease in the number of receptors for many peptide hormones [2]. It is well known that in man that the steroidogenic response to repeated ACTH administrations does not result in diminished cortisol secretion, but results in its enhancement [3, 4]. Similar findings were reported in Cushing's disease and in the ectopic ACTH syndrome [5, 6] as well as in rats bearing an ACTH-secreting tumor MtT-F4 [7]. On the other hand, desensitization has been demonstrated in adrenocortical cells in vitro with ACTH [8, 9], although it has not been shown to be associated with the decrease in its own receptor [10]. On the contrary, Penhoat et al. [11, 12] have recently revealed
that pretreatment with ACTH results in an increase in ACTH binding sites associated with enhanced cAMP response to further ACTH stimulation. These contradictory results prompted us to examine whether ACTH regulates its receptor at a pretranslational level in vivo in humans and in rats. The present study suggests that ACTH increases the levels of ACTH-R mRNA in normal adrenocortical cells.

**Materials and Methods**

**Patients**

Two patients with typical Cushing's syndrome were studied. The two patients presented the typical signs and symptoms of Cushing's syndrome such as moon face, buffalo hump, high blood pressure and amenorrhea. Patient A had multiple pathological fractures of thoracic vertebrae. Ultrasonography and computed tomography revealed right adrenal tumor in both patients. Their clinical findings and tumor sizes are summarized in Table 1. Hormones were measured with commercial radioimmunoassay kits. Corticotropin-releasing factor (CRF) test was performed by administering CRF [13] (100 µg) intravenously. Blood samples were obtained 0, 15, 30, 60, 90 and 120 min after the administration. ACTH test was performed in patient B by intramuscular administration of a 40 IU/2 ml/body of ACTH-Z1-24 (Cortrosyn-Z, Daiichi, Tokyo). Serum cortisol levels were determined before and 6 h after ACTH administration. The diurnal rhythm of the cortisol secretion was assessed by the determination of the cortisol level at 0800, 1200 1600 and 2100 h. In one patient (patient B), the administration of ACTH-Z resulted in an increase in cortisol from 33.1 to 102.0 µg/dl but no circadian rhythm of cortisol secretion or ACTH response to CRF test was observed in either patient. Adrenal tumor as well as adjacent adrenal tissue were resected by a retroperitoneal approach. Portions of resected adrenomas and adjacent non-neoplastic adrenal tissue were immediately frozen in liquid nitrogen and kept at -80 °C until RNA extraction. The rest of the adrenal tissue was histologically examined.

**Animals**

Male Wistar rats, weighing approximately 180 g,
were purchased from the Nippon Seibutsu Zairyo (Tokyo). The rats were housed in an air- and light-conditioned room maintained around 26 °C. Food and water were given ad libitum. Each group consisted of 4 rats. To all rats except the control group dexamethasone (Decadron, Banyu, Tokyo) was intraperitoneally administered at a dose of 400 µg/100 g body weight (BW) every 24 h for 5 days. ACTH (Cortrosyn, Daiichi, Tokyo, Japan; 25 IU/2 ml) was given intravenously at a dose of 5 IU/100 g BW, and the rats were killed at 15, 30, 60, 120, and 180 min after the ACTH. In other groups of dexamethasone-treated rats, ACTH-Z1-24 was administered intramuscularly and they were killed at 6, 12, 24, 48 and 72 h after ACTH-Z1-24. ACTH-Z1-24 and dexamethasone were administered once every 24 h at 1000 h. The rats were killed by decapitation and the adrenal glands were excised and cleaned of adherent fat. They were then weighed, frozen immediately in liquid nitrogen and stored at −80 °C for RNA isolation.

Preparation of cDNAs

Human ACTH-R cDNA was synthesized by RT-PCR (reverse transcription-polymerase chain reaction). One µg of total RNA isolated from the normal adrenal tissue adjacent to the adrenal adenoma of a patient A was reverse-transcribed with (dT)15 primer. The single stranded cDNA was used as a template for subsequent PCR by using a specific primer pair: sense: 5'-TGATTC-CATATCTTTGC-3' and antisense: 5'-TGGAGC-CAAGCAGGGAGAGG-3'. The sense primer was located at position -288 when the translation start site was designated as +1 [14]. The antisense was at position + 333. The PCR generated a fragment of cDNA of an expected size. After purification of the cDNA by electrophoresis through 1.5% agarose gels 15 µg after denaturation for 60 min at 56 °C in 1 M glyoxal, 50% (w/v) dimethylsulfoxide and 10 mM sodium phosphate buffer (pH 7.0). After the electrophoresis, the RNA was transferred from the gel to GeneScreen Plus (Biotechnology Systems NEN Research Products, Boston) according to the instructions of the manufacture. The membranes were hybridized with 32P-dCTP-labeled ACTH-R. The labeled probe was prepared with a Random Primed Labeling Kit (Boehringer, Mannheim, Germany). The procedures for hybridization and washings were described previously [17]. After washing the membrane, it was exposed to Kodak X-AR film (Eastman Kodak, Rochester, NY) at −80 °C. Intensities of autoradiographic bands were measured by densitometry with an image analyzer (TIB-100, Immunomedica Inc, Shizuoka, Japan). The same membrane was rehybridized with GAPDH cDNA to monitor the equal delivery of RNA samples.

Results

When total RNA samples extracted from the adenomas and the adjacent normal tissues were subjected to Northern blot analysis for ACTH-R mRNA, two different ACTH-R mRNA species with 4.0 kb and 2.0 kb were demonstrated (Fig. 1). In patient A, the mRNAs were only observed in the adenoma tissue. In patient B, they were present in both normal and adenoma tissue but the intensity
of the bands was much higher in the adenoma tissue. The intensity of the mRNA bands for GAPDH was similar in all the lanes (Fig. 1).

In the rat adrenal gland, ACTH-R mRNA was also demonstrated as 2 species with 4.5 kb and 2.0 kb in control rats (data not shown). Administration of dexamethasone resulted in a decrease in both ACTH-R mRNA species to an undetectable level in the adrenal gland. When ACTH was intravenously injected into the dexamethasone-treated rats, the receptor mRNA was not detected from 0.5 to 12 h after the injection (data not shown). However, the receptor mRNAs (both 4.5 kb and 2.0 kb) were detected 24 h after ACTH-Z administration (Fig. 2A). Figure 2B depicts the changes in the abundance of the two ACTH-R mRNAs corrected by that of GAPDH mRNA. The two receptor mRNAs increased in parallel, reaching a plateau at 48 h when ACTH-Z1-24 was administered every 24 h (Fig. 2B).

Discussion

The present study clearly demonstrated that ACTH-R mRNA in rat adrenal glands is increased by its own ligand. Penhoat et al. demonstrated that repeated treatment of bovine adrenocortical cells in culture with ACTH caused an increase in the number of ACTH binding sites without modifying the binding affinity [11]. Furthermore, they demonstrated that the increase in ACTH receptor required de novo protein synthesis and began at 24 h and reached its maximum at 48 to 96 h post-stimulation. This time course was concordant with the increase in ACTH-R mRNA in the present study. It has recently been shown in bovine and human adrenocortical cells cultured in vitro that ACTH-R mRNA was also increased by its own ligand [18-20]. These data suggest that ACTH increases its own receptor at least in part at a pretranslational level. Since the increase in ACTH-R after ACTH stimulation was shown to be
mimicked by 8-bromoadenosine 3', 5'-cyclic monophosphate, the mRNA increased by ACTH could be mediated by an increase in cAMP [11]. On the other hand, we have shown that ACTH increases the expression of c-fos and c-jun genes [21]. Their products, FOS and JUN, in turn could promote the transcription of ACTH-R gene via AP-1 site. However other possibilities, such as the stabilization of mRNA proposed by Penhoat [19], cannot be ruled out.

Two different sizes of ACTH-R mRNA (with 4.5 kb and 2.0 kb in rats and 4.0 kb and 2.0 kb in man) were observed. This finding is compatible with the results obtained by Lenhox et al. [22] for the rat and Mountjoy et al. [14] for the human adrenal gland. It was reported that the predominant mRNA species for ACTH-R is 4.0 kb in human adrenal glands but the intensity of the two mRNA species is quite similar in the rat adrenal tissue and the normal human adrenal gland of patient B. The smaller mRNA was more predominant in the two adenoma tissues from both patients. It is unknown whether the differences in the abundance of the two mRNA species in the adenoma could be affected by cortisol excess or by ACTH. Since it is known that the ACTH-R gene has no intron [14], the two mRNA species may be produced by alternative initiation or alternative polyadenylation.

The absence or decreased level of ACTH-R mRNA in the normal adjacent adrenal tissues from the two patients with Cushing’s syndrome might be explained as the consequence of the inhibition of ACTH secretion since decreased ACTH-R mRNA caused by dexamethasone treatment in the rat was restored by ACTH. This notion is supported by the findings obtained by Kolanowski et al. [3, 4], since they demonstrated that increased steroidogenic response to repeated ACTH administration could be observed not only in patients treated with excess glucocorticoid but also in those with anterior hypophyseal insufficiency.

The existence of ACTH-R mRNA in the two adenoma tissues was surprising. The observation that the increase in the cortisol level due to ACTH in patient B could indicate that ACTH-R expressed in the adenoma is functional. This assumption was supported by the findings of Lamberts et al. [23] and Mizuno et al. [24]. They showed that ACTH stimulated cortisol secretion by isolated adenoma cells from some patients with Cushing’s syndrome. Parma et al. recently demonstrated that somatic mutations in the TSH receptor gene could cause hyperfunctioning thyroid adenoma [25]. This result raises the possibility that ACTH-R expressed in the adenoma tissue could be related to the development of autonomous synthesis and secretion of steroid hormones.

References


