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ACTH Increases Expression of *c-fos, c-jun* and β-actin Genes in the Dexamethasone-treated Rat Adrenals

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Abstract. Our recent finding that ACTH increases *c-fos* mRNA in the adrenal gland of hypophysectomized rats indicates that the gene product FOS may play an important role(s) in mediating the action of ACTH. However, hypophysectomy employed in that study causes the disappearance of trophic hormones other than ACTH and may modify the effect of ACTH. Thus, in the present investigation, dexamethasone-treated rats were used. Since FOS functions only when it dimerizes with JUN (the product of *c-jun* gene), the changes in the levels of *c-fos* and *c-jun* mRNAs were studied together with that of β-actin mRNA which is also affected by ACTH. Northern blot analysis was employed to determine the mRNA levels. It was demonstrated that ACTH increases the mRNAs coding *c-fos* and *c-jun* in the adrenal glands of dexamethasone-treated, ACTH-suppressed rats. The *c-fos* mRNA was not detectable before ACTH administration. After ACTH administration, the mRNA levels were transiently increased, the maximum level being observed at 30 min after ACTH. At 180 min post ACTH, the level returned to the unstimulated level. The mRNA coding *c-jun* was detectable before ACTH administration and it also increased rapidly after ACTH with maximal stimulation at 30 min. However, the mRNA level at 180 min post ACTH was still higher than the unstimulated level. The changes in β-actin mRNA were approximately the same as those of *c-jun* mRNA. These results suggest that increased expression of *c-fos*, *c-jun* and β-actin genes by ACTH may play an important role in mediating its action on the adrenals.

Key words: *c-fos*, *c-jun*, β-Actin, Rat adrenal, ACTH. (*Endocrinol Japon* **39**: 377–383, 1992)

ACTH stimulates steroidogenesis of the adrenocortical cells and their proliferation. When rats are hypophysectomized or ACTH secretion from their pituitary is suppressed by dexamethasone, the adrenals undergo atrophy. ACTH administration to the animals results in increased secretion of corticosterone and hypertrophy of the adrenocortical cells. It has been shown that ACTH also stimulates the transcription of mRNAs coding for steroidogenic enzymes (cytochrome P-450) and promotes DNA synthesis [1–5]. However, the mechanism involved in these ACTH-mediated effects has not been elucidated.

Recently, we showed that ACTH transiently increases *c-fos* mRNA in the adrenal gland of hypophysectomized rats and suggested that the product of the *c-fos* gene [FOS] may play an important role(s) in mediating ACTH action [6]. Since FOS has been shown to dimerize with JUN (a product of the *c-jun* gene) and transactivates gene expression by binding to the specific DNA sequence [7–10], changes in the expression of the *c-jun* gene were studied in the present investigation. Hypophysectomy employed in the previous study

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impairs the secretion of not only ACTH but also other trophic hormones and could modify the effect of ACTH on adrenocortical cells. Thus, in this study, dexamethasone-treated rats were used instead of hypophysectomized rats.

Materials and Methods

Animal treatment

Male Wistar rats, weighing approximately 180 g, were purchased form the closed colony of the Shizuoka Experimental Animal Agricultural Cooperative Union (Hamamatsu, Shizuoka, Japan). The rats (four per cage) were maintained under controlled temperature (26°C) and lighting (12 h of light and 12 h of darkness). Rat pellets and water were given *ad libitum*.

Each group consisted of four rats. To all rats except the control group, dexamethasone (Decadron, Banyu, Tokyo, Japan; 8 mg/2 m*l*) was intraperitoneally administered at a dose of 4 mg/kg body weight (BW) for three days (Fig. 1).

As shown in Fig. 1, ACTH (Cortorosyn, Daiichi, Tokyo, Japan; 25 IU/2 m*l*) was given intraperitoneally at a dose of 50 IU/kg BW. The rats were killed at 5, 15, 30, 60 and 180 min after the injection of ACTH.

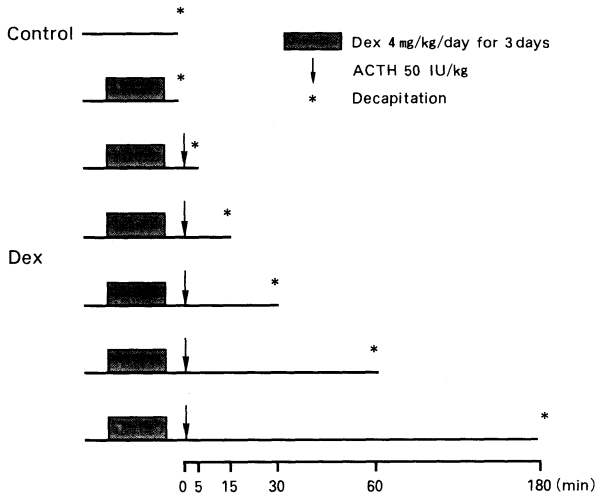

Fig. 1. Experimental protocol. To all rats except the control group, dexamethasone was administered intraperitoneally at a dose of 4 mg/kg BW for 3 days. Twenty-four hours after the final dexamethasone, ACTH (50 IU/kg BW) was injected intraperitoneally. Then, the rats were killed at 5, 15, 30, 60 and 180 min.

Rats were killed by decapitation and trunk blood was collected for later determination of corticosterone. The adrenal glands were excised and cleaned of adherent fat, weighed, frozen immediately in liquid nitrogen and stored at −80°C for RNA isolation.

Preparation of RNA

Total RNA was isolated in a single step extraction by an acid guanidium thiocyanate phenol chloroform method [11]. The amount of RNA was estimated by optical density at 260 nm. The ratio of absorption at 260 versus 280 nm was always greater than 1.75.

Probes

Human *c-fos* cDNA [12], human *c-jun* cDNA [13] and chicken β-actin cDNA [14] were used as probes. The respective cDNA inserts were purified by electrophoresis after digestion with appropriate restriction enzymes. Purified cDNAs were labelled with [α-^{32}P]dCTP (SA, 3,000 Ci/mmol; New England Nuclear, Boston, MA) to about 10^8 cpm/μg, using a random primed DNA labelling kit (Boehringer Mannheim, Germany) [15].

Analysis of mRNA

Northern blot hybridizations were employed for analysis of mRNAs. Twenty μg of RNA extracted from the adrenal glands of rats was subjected to electrophoresis through 1% (w/v) agarose gels after denaturation for 60 min at 50°C in 1 M glyoxal, 50% (v/v) dimethylsulphoxide and 10 mM phosphate buffer (pH 7.0). After electrophoresis, the RNA was transferred from the gel to a Magna Graph Membrane (New England Nuclear, Boston, MA, U.S.A.) according to the manufacture's instructions [16]. The membrane was prehybridized in a solution containing 5 × SSPE (1 × SSPE = 180 mM NaCl, 1 mM EDTA and 10 mM Sodium phosphate; pH 7.4), 5 × Denhardt's solution (1 × Denhardt's solution = 0.02% ficoll, 0.02% (w/v) polyvinylpyrrolidone and 0.02% (w/v) bovine serum albumin (fraction V, crystallized and globulin free; Sigma, St Louis, MO, U.S.A.)), 0.1% (w/v) sodium dodecylsulphate (SDS), herring sperm DNA (0.1 μg/m*l*; Boehringer-Mannheim, Mannheim, Germany) and 50% (v/v) formamide

(Merck, Darmstadt, Germany). Hybridization was performed at 42°C for 20 h in a solution containing 5 × SSC (1 × SSC = 150 mM NaCl plus 15 mM sodium citrate; pH 7.0), 2 × Denhardt's solution, 0.1% SDS, herring sperm DNA, 50% (v/v) formamide and ^{32}P-labelled denatured probes (10^6 cpm/m*l* buffer).

The membranes were washed at room temperature twice in 2 × SSC-0.5% SDS for 15 min, then at 50°C twice for 15 min in 1 × SSC-0.5% SDS and finally at room temperature twice for 15 min in 0.1 × SSC-0.1% SDS. The membranes were exposed for 5 days (*c-fos*), 4 days (*c-jun*), 1 day (β-actin) to Kodak X-AR film at −70°C. When the membranes were used for rehybridization with other probes, pre-existing ^{32}P-labelled probe on the membranes was removed by boiling the membranes in 0.1 × SCC-0.1% SDS for about 2 h. Intensities of autoradiographic bands were measured by densitometry with an image analyzer (TIB-100, Immuno Medica Inc, Shizuoka, Japan).

Corticosterone assay

Serum corticosterone was determined by radioimmunoassay with rabbit anti-corticosterone antibody obtained from Teikoku-Zoki (Tokyo, Japan) [17].

Statistical analysis

All values were expressed as the mean ± SD. Statistical significance was assessed by unpaired Student's *t*-test.

Table 1. Changes in body weight and adrenal weight after dexamethasone treatment and ACTH administration

	Body weight (g)	Adrenal weight (mg/2 glands)
Control	253.3±8.76	42.0±3.67
0 min	212.0±4.73**	33.4±2.51*
5 min	206.1±6.94**	33.7±2.88*
15 min	206.6±7.53**	30.4±2.07**
30 min	209.2±8.01**	27.6±2.19**
60 min	214.2±6.65**	26.2±5.23**
180 min	201.7±4.08**	33.0±5.10

Values are expressed as the means±SD.**, $P<0.001$ *vs.* control; *, $P<0.01$ *vs.* control.

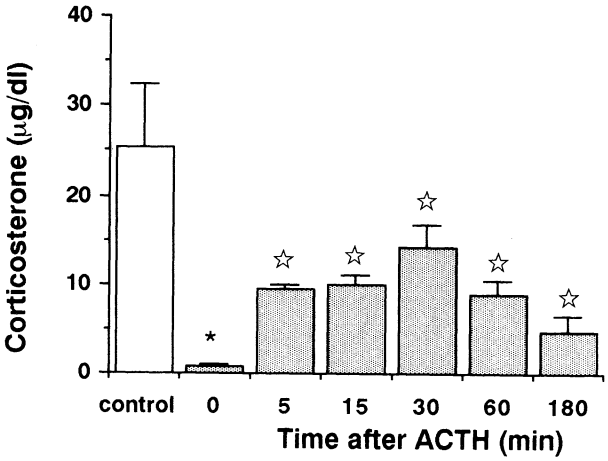

Fig. 2. Changes in the serum corticosterone levels following dexamethasone-treatment and ACTH administration. The data are expressed as the means ± SD. *, $P<0.001$ *vs.* control; ☆, $P<0.001$ *vs.* 0 time.

Results

As shown in Table 1, body weights and adrenal weights were significantly decreased by administration of dexamethasone for three days. ACTH administration did not cause significant changes in these weights during the period (180 min) studied.

The serum corticosterone level in the control group was 25±7 μg/d*l*, while the level in the dexamethasone-treated group was 0.8±0.1 μg/d*l*, suggesting that endogenous secretion of ACTH was effectively suppressed by dexamethasone. ACTH administration to the dexamethasone-treated rats resulted in a rapid increase in corticosterone (Fig. 2). A significant increase was observed as early at 5 min after ACTH.

As shown in Fig. 3-A, *c-fos* mRNA was observed as a single band of 2.2 kb on Northern blot. The mRNA size was similar to those reported previously [18]. Densitometric analysis of the mRNA band is shown in Fig. 3-B. The level of *c-fos* mRNA at 0 and 5 min after ACTH was undetectable. It became detectable at 15 min after ACTH and peaked at 30 min, decreasing to an undetectable level at 180 min.

As shown in Fig. 4-A, *c-jun* mRNA was detected as double bands: 2.7 and 3.2 kb. This observation was compatible with previous reports [19, 20]. Note that the changes in the density of the two bands were similar. Thus, the two bands were

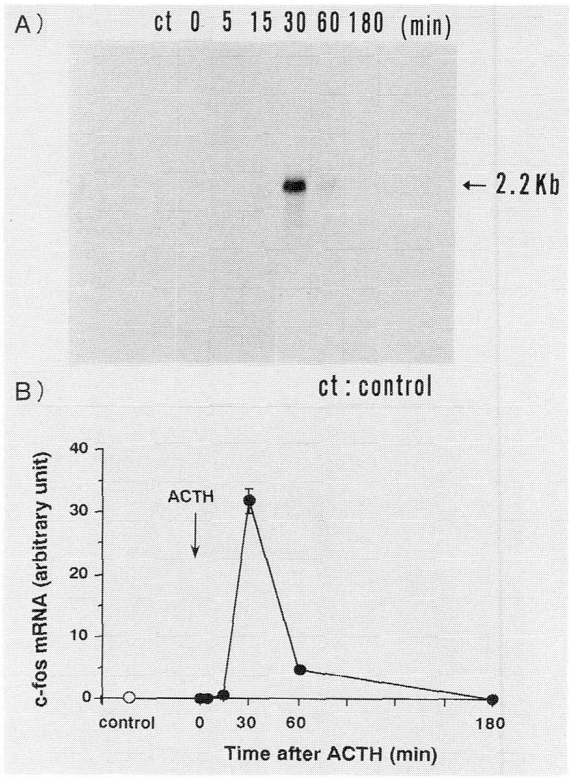

Fig. 3. Effect of dexamethasone-treatment and ACTH administration on adrenal *c-fos* mRNA. (A) Total RNA (20 μg/lane) was electrophoresed, and Northern blot hybridization with *c-fos* cDNA was performed. A single 2.2 kb band of *c-fos* mRNA was detected. (B) The intensity of the specific bands for *c-fos* mRNA from all of the rats was measured by densitometry and is shown as arbitrary units. The data are expressed as the means ± SD.

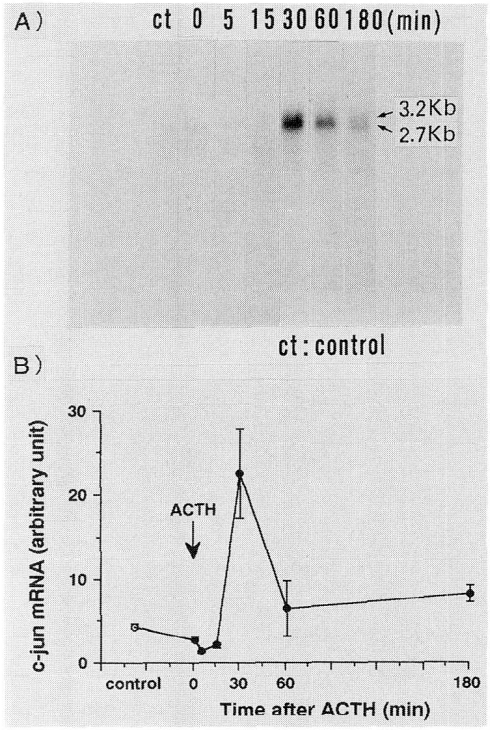

Fig. 4. Effect of dexamethasone-treatment and ACTH administration on adrenal *c-jun* mRNA. (A) The procedure was the same as that described in Fig. 3 except that the membrane was hybridized with *c-jun* cDNA. Two bands of *c-jun* mRNA were observed, one at 2.7 kb and the other 3.2 kb. (B) The intensity of the bands for *c-jun* mRNA expressed as arbitrary units is shown.

collectively analyzed by densitometry. Dexamethasone administration for three days did not cause a significant change in *c-jun* mRNA. Administration of ACTH rapidly increased the mRNA level with a peak at 30 min. Although decreased, the amounts of *c-jun* mRNA at 60 and 180 min after ACTH were greater than at 0 min.

β-actin mRNA was detected as a single 2.2 kb band (Fig. 5-A). The mRNA amount did not change following dexamethasone administration. ACTH administration to dexamethasone-treated rats also resulted in a rapid increase in the mRNA with a peak level at 30 min after ACTH. At 60 and 180 min after ACTH, the level was still significantly higher than at 0 min.

Discussion

We have recently shown that ACTH induces the expression of the *c-fos* gene in the adrenal gland of hypophysectomized rats and indicated that the gene product FOS may play an important role(s) in mediating the action of ACTH. In the present investigation, dexamethasone-treated rats were used since the disappearance of trophic hormones other than ACTH by hypophysectomy may modify the effect of ACTH. Also, the changes in *c-jun* mRNA caused by ACTH were studied, since FOS functions only when it dimerizes with JUN (the product of the *c-jun* gene) [21, 22].

The time course of ACTH-induced changes in *c-fos* mRNA in the adrenal glands of dexamethasone-treated rats was similar to our previous observation in hypophysectomized rats [6]. The result indicates that short-term elimination of

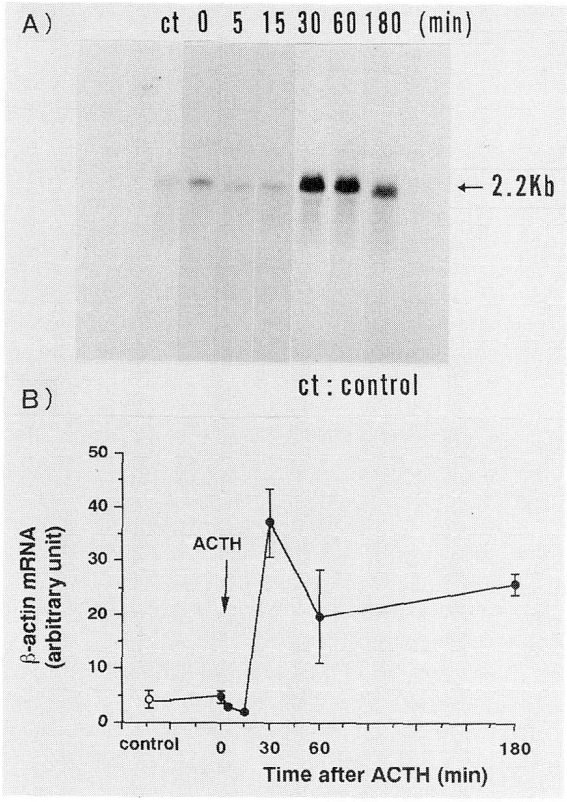

Fig. 5. Effect of dexamethasone-treatment and ACTH administration on adrenal β-actin mRNA. (A) A single band of β-actin mRNA was observed at 2.2 kb. (B) The intensity of the band for β-actin mRNA is shown.

other trophic hormones by hypophysectomy does not affect the ACTH action on *c-fos* gene expression.

Simultaneous determination of changes in *c-fos* and *c-jun* mRNAs revealed that ACTH induces both mRNAs with similar time courses. This finding is compatible with the idea that FOS and JUN function in combination. A concomitant increase in *c-fos* and *c-jun* mRNA has been shown in response to the addition of EGF (epidermal growth factor) to Rat-1 cells [23] and growth hormone to 3T3-F442A cells [19].

Although the time courses were similar, the changes in the abundance of these mRNA were somewhat different. The abundance of *c-fos* mRNA was undetectable before stimulation with ACTH while that of *c-jun* was detectable. The decrease in *c-fos* mRNA was more rapid than that of *c-jun* mRNA. These results may indicate the difference in the turnover rate of the two mRNAs. Also expression of the *c-jun* gene may be necessary for the basal activity of adrenal cells. It has been shown that the homodimer of JUN is functional [24, 25].

Although the present investigation could not answer the question how ACTH increases *c-fos* and *c-jun* mRNAs, we have recently shown that the ACTH-induced-increase in cAMP is responsible for the induction of *c-fos* mRNA [26]. *c-jun* mRNA has also been shown to be induced by cAMP [27]. The induction of JUN may increase *c-jun* mRNA as suggested by Anjel *et al.* [28]. This may be one of the explanations for the sustained increase in *c-jun* mRNA after ACTH.

The physiological significance of ACTH-induced changes in *c-fos*, *c-jun* and β-actin mRNA remains to be elucidated. Involvement in the acute action of ACTH, the mobilization of cholesterol from its storage site to the inner mitochondrial membrane and removal of the side chain of cholesterol [3], seems to be unlikely since the step can be activated in seconds. Thus, ACTH-induced changes in *c-fos*, *c-jun* and β-actin mRNA may be involved in the chronic action of ACTH which is characterized by the increased synthesis of DNA, RNA and steroidogenic cytochrome P-450 enzymes. The FOS-JUN complex has been shown to play an important role in cell proliferation [19, 21, 22, 29]. β-actin also participates in the proliferation. It has been shown that 5'-upstream of the bovine cytochrome P-450scc gene contains a sequence similar to the FOS-JUN binding site (AP1-site) [30]. Thus, ACTH-induced FOS-JUN may be involved in the activation of P-450scc.

Although the role of FOS and JUN in mediating ACTH action is a matter of speculation, a recent immunohistochemical observation that the changes in the levels of FOS protein in rat adrenals follow the circadian rhythm of ACTH [31] support the idea that they may play a physiological role in mediating the action of ACTH.

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