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Effect of ACTH on the Proliferation of the Rat Adrenal Gland

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Abstract: Although the regulation of steroidogenesis in adrenal fasciculata and reticularis is stimulated by adrenocorticotrophic hormone (ACTH), its role on the proliferation of adrenocortical cell is not established. We thus investigated the effect of ACTH on the proliferation of adrenocortical cells by analyzing the expression of proliferating cell nuclear antigen (PCNA). Endogeneous secretion of ACTH was inhibited by treatment with dexamethasone for 5 days. At intervals after ACTH treatment, homogenate of the adrenal gland was subjected to Western blot analysis. PCNA expression was detected in control rat without any treatment. Dexamethasone administration for 5 days resulted in the reduction of PCNA expression. ACTH administration to the dexamethasone treated rats increased PCNA expression at 24 hr. It is thus demonstrated that ACTH increases cellular proliferation in aderenal cortex.

Key words: ACTH, PCNA, adrenal gland, proliferation

ACTH is the major regulator of steroidgenic function of the adrenocortical zonae fasciculata and reticularis (Simpson, 1983) (Gill, 1972). ACTH not only acts on the immediate, transcription-independent stimulation of adrenal steroid synthesis and release, but also increases the expression of a number of genes including those involved in steroidogenesis. It is well established that hypophysectomy decreases, while ACTH treatment increases adrenal gland volume, suggesting its role in cellular proliferation. However, ACTH was found to behave as an antimitogenic hormone in certain cell lines derived from adrenal cortex (Forti, 2002).

In this study, we examined the effect of ACTH on the proliferation of the rat adrenal gland by Western blot analysis using an antibody against proliferating cell nuclear antigen (PCNA).

Materials and Methods

1. Animal treatment

The experimental protocol was approved by the Committee for Animal Experiment of the Research Institute of Environmental Medicine, Nagoya University. Male Wister rats, weighing approximately 180 g, were maintained under controlled temperature (23 °C) and lighting (12-h light / 12-h dark cycle) with access to food and water *ad libitum*. Each group consisted of six rats. To all rats except the control group, dexamethasone (Decadron, Banyu, Tokyo, Japan) was administered intraperitoneally once a day at a dose of 4 mg/kg BW for 5 days. After dexamethasone treatment, ACTH (Cortrosin, Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan) was injected intramusclarly once a day at a dose of 50 IU/kg BW. The rats were killed by decapitation at 12, 24, 48, and 72 hr after ACTH injection. The adrenal glands were excised, cleaned of fat, weighed, frozen immediately in liquid nitrogen, and stored −80 °C until total protein extraction.

2. Protein extraction

The tissue fragments were washed in phophate-buffered saline (PBS) at 0 °C. After centrifugation at 3,000 xg for 5 minutes at 4 °C, all of the supernatant were removed by aspiration. The tissue fragments were dispersed in 5 volumes of ice-cold suspension buffer (0.1M NaCl, 0.01M tris-HCl (pH7.6), 0.001M EDTA (pH8.0), Protease Inhibitor Cocktail Tablets (complete, Mini, EDTA-free; Roche, Mannheim, Germany). Equal volume of 2 × SDS gel-loading buffer was added. The samples were placed in a boiling-water bath for 10 minutes and sonicated for 2 minutes to shear the chromosomal DNA. After centrifugation at 10,000 xg for 10 minutes, the supernatant was used for Western blot analysis. The protein concentration in the supernatant was determined by Bradford method using a kit from BioRad (Hercules, California, USA)

3. Western blot analysis

Volume of the sample containing 50 mg of protein was adjusted to 20 ml with 1 × SDS gel-loading buffer and boiled for 3 minutes, electrophoresed on 12% polyacrylamide gels in

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the presence of SDS, and electroblotted onto polyvinylidene difluoride membranes (Hybond-P; Amersham Biosciense, Amersham, England) using a semidry Bio-Rad apparatus. The membrane was incubated for 1 hr with blocking buffer [TBST (10 mM Tris-HCl, pH7.4, 150 mM NaCl, 0.1% Tween-20) containing 5% bovine serum albumin (BSA) (Fraction V; SIGMA, St. Louis, Missouri, USA)]. Then the membrane was incubated for 16 hr with a mouse monoclonal antibody specific for PCNA (BD Transduction Laboratories, San Jose, California, USA) diluted at 1:2,000 with TBST. After washing three times with TBST, it was incubated for 2 hr with anti-mouse IgG conjugated with horseradish peroxidase (SIGMA, St. Louis, Missouri, USA) diluted at 1:10,000 with TBST. After washing three times, the membrane was incubated with a substrate for horseradish peroxidase (SuperSignal West Pico Chemiluminescent Substrate, PIERCE, Rockford, Illinois, USA) for 5 minutes. It was detected by Bio/chemical-luminescence photography (Light Capture; ATTO, Tokyo, Japan).

Results and Discussion

Effect of ACTHon growth regulatory mechanisms of adrenocortical cells remains essentially unknown. In Y1 mouse adrenocortical cell line, ACTH inhibited cell cycle progression (Lotfi, 1997). But in vivo, ACTH withdrawal induced adrenocortical apoptosis (Wyllie, 1973). ACTH reduced apoptosis in the adrenal cortex of hypophysectomized rats (Ceccatelli, 1995).

PCNA is a 36 kDa protein consisting of 262 amino acids. It is the polymerase d-subunit necessary for DNA synthesis or repair. PCNA protein level peaks during the S-phase of the cell cycle. It is almost undetectable in other phase of the cell cycle. Because of its unique expression, PCNA has been extensively used to study the prognosis of tumor progression and neoplastic proliferation.

Male Wister rats were treated with dexamethasone to suppress endogenous ACTH secretion. As shown in Fig. 1. PCNA was demonstrated as a major band at 36 kDa on the immunoblot of the control group. Treatment with dexamethasone for 5 days reduced the expression. Subsequent treatment with ACTH increased the level of PCNA at 24 hours after the administration, followed by a gradual increase until 72 hr. Abundant expression of PCNA in non-treated control suggests that adrenal undergoes constant regeneration. Considering the effect of ACTH on zonae fasciculata and reticularis, it is speculated that cellular proliferation is mainly in these zonae. However, PCNA staining in control rat was shown to be strongest in zona reticularis, suggesting the stem cell may be present in this zone (Wolkersdorfer, 1996). Further histological study is necessary to ascertain the origin of proliferating cells.

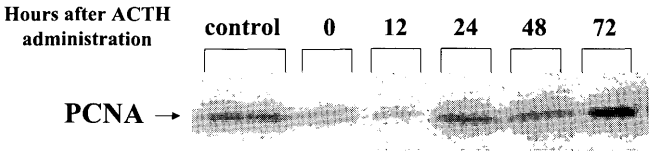

Fig. 1 Western blot analysis. PCNA was demonstrated as a band at 36 kDa. Treatment with dexamethasone reduced the expression. Subsequent treatment with ACTH increased the level of PCNA at 24 hours after the administration, followed by a gradual increase until 72 hr.

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