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Long-Term Exposure to High Corticosterone Levels Inducing a Decrease of Adenylate Kinase 1 Activity*

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Abstract: Corticosterone, a principal glucocorticoid synthesized in the rodent adrenal cortex, can be cumulatively toxic to hippocampal neurons, the cause of which is not known. The present study determined whether the cytosol adenylate kinase (AK) system was involved in the neuronal damage induced by long-term exposure to high corticosterone levels. We investigated the effects of long-term exposure to high corticosterone levels on AK1 activity, AK1 mRNA expression, and energy levels in cultured hippocampal neurons. The results show that long-term exposure to high corticosterone levels induces a reduction of the cultured hippocampal neuron viability, significantly reduces energy levels, and causes a time-dependant reduction of the AK1 activity. These findings indicate that changes in the AK system might be the mechanism underlying neuronal damage induced by long-term exposure to high corticosterone levels.

Key words: corticosterone; adenylate kinase; adenine nucleotide; neuron; cell viability

Introduction

Corticosterone is a principal glucocorticoid synthesized in the rodent adrenal cortex and secreted in response to stress. A series of studies indicate that corticosterone can be cumulatively toxic to hippocampal neurons. For example, exogenous chronic application of a high dose of corticosterone results in time-dependent neuronal damage, ranging from an initial and reversible atrophy of dendritic processes to the irreversible loss of hippocampus pyramidal cells[1-3]. Yu et al.[4] found that high levels of corticosterone significantly suppressed the proliferation of fetal hippocampal progenitor cells, decreased the number of bromodeoxyuridine-labeled neurons, and caused dendritic atrophy in microtubule-associated protein 2-labeled neurons *in vitro*.

Regarding the molecular mechanisms underlying the detrimental actions of corticosterone on hippocampal neurons, Jani et al.[5] found that chronic corticosterone treatment resulted in uncoupling of mitochondrial oxidative phosphorylation. Furthermore, chronic corticosterone treatment induced a decrease of the mitochondrial volume fraction in the hippocampal area CA3 of rats[6] and resulted in drastic impairment of ATP synthesis rates in the brain mitochondria of rats, as reflected by the lowering of ADP phosphorylation rates[7]. These studies suggested that changes in intracellular

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energy metabolism might be involved in the neuron damage induced by long-term exposure to high corticosterone levels.

The adenylate kinase (AK, EC 2.7.4.3) system is also known to play an important role in intracellular energy metabolism[8]. AK is an evolutionary conserved family of enzymes that catalyzes the reversible reaction of ATP + AMP = 2ADP and is responsible for buffering the ATP/ADP ratio and energy transfer[9]. Consequently, AK prevents marked increases in the ATP/ADP ratio at the site of energy generation and marked decreases in the ratio at the site of ATPase, which doubles the efficiency of the diffusion of ATP in the transfer of energy and maintains efficient intracellular energy flow[10,11]. Recently, a series of studies also showed that AK regulated the membrane K^+-ATP channel[12] and ATP-binding cassette (ABC) transporter activity[13], and was required for extracellular ATP synthesis[14]. Thus, abnormalities of the AK system may produce detrimental effects on cell or tissue functions.

Three AK isozymes (AK1, AK2, and AK3) have been characterized in vertebrates. AKl is present in the cytosol of skeletal muscle, brain and erythrocyte. AK2, which is undetectable in the brain[15], is found in the intermembrane space of the mitochondria of liver, kidney, spleen, and heart. AK3, correctly called GTP:AMP phosphotransferase, exists in the mitochondrial matrix of the liver and the heart[16].

This research tests the hypothesis that long-term exposure to high corticosterone levels results in the decrease of AK1 activity in the cytosol of hippocampal neurons. Reduced activities of AK1 give rise to relatively lower energy levels at the site of energy consumption, which may produce detrimental effects on neuronal functions.

1 Materials and Methods

1.1 Cultivation of hippocampal neurons from newborn rat brain

The animals were treated according to the *Guidelines on Accommodation and Care of Animals* formulated by the Chinese Convention for the protection of vertebrate animals used for experimental and other scientific purposes. Brains were removed from newborn Sprague-Dawley rats (Laboratory Animal Institute, Chinese Academy of Medical Sciences, Beijing, China) and maintained in phosphate buffered saline (PBS) (pH ≈ 7.4) at 4℃. Hippocampi was dissected from the brains and placed into a vial containing 2 mL of cold PBS. Then, the tissue was minced into small pieces and incubated at 37℃ in a 0.1% trypsinase (Sigma) solution for 15 min. After termination of the trypsinization, the tissue was dissociated by trituration with a fire-polished pipette. After dispersion, the cells were filtered through a 90-μm wire mesh screen into a 10-mL centrifuge tube, and then centrifuged for 5 min at 1000 r/min. The pellet was resuspended gently in Dulbecco's modified Eagle's medium (DMEM, Gibco) with 10% fetal bovine serum (FBS, Hyclone) and the neuronal cell concentration adjusted to about 1×10^6 cells/mL with 1 mL inoculated into each well of a 24-well plate (Costar) precoated with poly-*D*-lysine (50 μg/mL; M_r > 300 000; Sigma).

The cells were cultured in serum-supplemented medium and maintained at 37℃ and 5% CO_2 for 45 min to initiate attachment of neurons to the substratum and to inactivate proteolytic enzymes released from damaged cells during trituration. After this initial attachment period, the medium and unattached cells were removed and replaced by the serum-free medium. The medium formulations were made by DMEM with the addition of penicillin/streptomycin (1 U/mL∶1 μg/mL; Amresco), 10 mmol/L HEPES (Sigma), 1 mmol/L pyruvic acid sodium (Sigma), 100 mg/L bovine serum albumin (BSA, Sigma), and N2 medium supplements. The N2 medium supplements were comprised of (final concentrations): glucose (6 g/L), insulin (10 mg/L), transferrin (20 mg/L), putrescine (62 μmol/L), progesterone (20 nmol/L), and sodium selenite (30 nmol/L) (media supplements obtained from Sigma). One-half of the culture medium was changed every 3-4 days. After 7- to 8-day primary cultures, the hippocampal neurons were continually treated with normal (0.1 μmol/L) or high levels (0.5 or 1 μmol/L) of corticosterone. Cell viability experiments and neurochemical measurements were performed at days 1, 3, and 5.

1.2 Cell viability

The hippocampal neuron viability was assessed by using neuron-specific enolase (NSE)-ELISA methods[17]. The cultured hippocampus neurons in each well of a 24-well plate were washed three times with cold