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Research Report

Chronic corticosterone injections induce a decrease of ATP levels and sustained activation of AMP-activated protein kinase in hippocampal tissues of male mice

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ABSTRACT

Chronic corticosterone injections induce hippocampus tissue damage and depression-like behavior in rodent animals, the cause of which is not known. Nevertheless, increasing evidence shows that adenylate kinase (AK) and AMP-activated protein kinase (AMPK) play a very important role in intracellular energy metabolism and are especially critical for neurons which are known to have very small energy reserves and narrow margin of safety between the energy that can be generated and the energy required for maximum activity. Abnormalities of AK or AMPK system have detrimental effects on neurons or brain function especially at times of increased activity. In this study, we investigated the effects of chronic corticosterone exposure on energy metabolism, as well as AK and AMPK in hippocampal tissues in male C57BL/6N mice. Our results show that chronic corticosterone injection induced depression-like behavior in male mice, significantly decreased the energy levels and caused a sustained increase of AMP:ATP ratio in hippocampal tissues. Interestingly, chronic corticosterone injections did not produce obvious effects on AK1 protein and mRNA levels, but caused a sustained activation of AMPK. The results indicate that sustained AMPK activation might be a mechanism by which chronic corticosterone treatment causes depression-like behavior in male mice.

1. Introduction

Corticosterone is a principal glucocorticoid synthesized in the rodent adrenal cortex and secreted in response to stress. There is increasing evidence that long-term exposure to high corticosterone levels produces detrimental effects on hippocampal neurons (Sapolsky et al., 1985; Woolley et al., 1990; Jacobson and Sapolsky, 1991; Mizoguchi et al., 1992; López et al., 1998; Duman et al., 1999; Karten et al., 1999), impairs hippocampal long-term potentiation (LTP) (Kim et al., 1996; Xu et al., 1997; Martin et al., 2000) and decreases spatial learning abilities of C57BL/6J mice (Grootendorst et al., 2002).

Recently, abnormalities of energy metabolism have been suggested to explain the corticosterone-induced hippocampus damages. For example, glucocorticoids could accelerate ATP loss, following metabolic insults in cultured hippocampal neurons (Lawrence and Sapolsky, 1994). *In vivo*, chronic corticosterone treatment induced a decrease of mitochondrial volume fraction in hippocampal area CA3 of rats (Anderson, 2004) and resulted in drastic impairment of ATP synthesis rates in the brain mitochondria of rats, as reflected by lowering of ADP phosphorylation rates (Katyare et al., 2003). As we know, central nervous system (CNS) cells need large amounts of energy. The great majority of energy used by CNS cells is for

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processes that subserve physiological functioning, some of which are even sensitive to small reductions in ATP (Hibbard et al., 1987). Consequently, abnormalities of energy metabolism may produce detrimental effects on neuro-physiological functions.

It is well known that energy metabolism involves mainly energy synthesis and energy metabolism. The latter mainly consists of adenylate kinase and an AMP-activated protein kinase system. Adenylate kinase (AK, EC 2.7.4.3) is an evolutionary conserved family of enzymes that catalyzes the reversible reaction of ATP+AMP=2 ADP (Russell et al., 1974). The reaction catalyzed by AK prevents the marked increase in the ATP/ADP ratio that would otherwise occur at the site of ~P generation, and the marked decrease in the ratio at the site of the ATPase.

AMP-activated protein kinase (AMPK) is a member of a larger metabolite-sensing protein-kinase family. It is a αβγ heterotrimer. AMPK activity depends on phosphorylation by an upstream kinase on Thr172 in the activation loop of the α subunit, and both phosphorylation and dephosphorylation are sensitive to AMP (Hawley et al., 1996). The available evidence indicates that AMPK plays a critical role in the regulation of cellular processes which are controlled by alterations in the energy state of cells and tissues. Activation of AMPK leads to phosphorylation of serine and threonine residues in key enzymes controlling cholesterol and fatty acid synthesis and results in their inhibition and ATP conservation. On the other hand, it favors fatty acid oxidation and stimulates glycolysis by increasing glucose transport and activating 6-phosphofructo-2 kinase, thereby favoring ATP production (Carling, 2004).

Nowadays, increasing evidence shows that energy regulation also plays a very important role in the organization of energy metabolism and maintenance of energy homeostasis within neurons, due to the fact that the brain has very small energy reserves, and the margin of safety between the energy that can be generated and the energy required for maximum activity is also small (Ames, 2000). Based on above rationale, here we test the hypothesis that reduced energy levels and energy deregulation might be responsible for corticosterone-induced behavioral abnormalities.

2. Results

2.1. *Chronic corticosterone injections induce depression-like behavior*

After chronic corticosterone treatments, depression-like behavior of mice were assessed by using forced swimming tests and tail suspension tests at weeks 1, 3 and 5, respectively. We found that 3-week and 5-week corticosterone injections significantly increased depression-like behavior of mice in both tests. However, the 1-week corticosterone injection was not found to increase but to decrease depression-like behavior. The statistical details of these observations are given below:

In the forced swimming test, there was a significant effect of time ($F_{2,84}=5.473$; $P<0.01$) and treatment on immobile time ($F_{1,84}=3.921$; $P<0.05$), as well as a significant interaction between treatment and time ($F_{2,84}=8.638$; $P<0.001$). Multiple comparison tests further showed that corticosterone significantly increased immobile time of mice at week 3 ($P<0.05$) and week 5 ($P<0.05$), but not at week 1 (Fig. 1a). One-week corticosterone treatment even tended to decrease immobile time of mice.

In the tail suspension test, two-way ANOVA also showed significant main effects of treatment ($F_{1,84}=5.454$; $P<0.05$) and time ($F_{2,84}=3.901$; $P<0.05$) with a significant interaction between these factors ($F_{2,84}=4.929$; $P<0.05$). Multiple comparison tests further revealed that the immobile time of mice treated with corticosterone decreased significantly at week 1 ($P<0.05$), but increased significantly at week 3 ($P<0.05$) and week 5 ($P<0.05$) (Fig. 1b).

2.2. *Chronic corticosterone injections induce a decrease of ATP levels and an increase of AMP levels in hippocampal tissues*

In order to determine whether chronic corticosterone injections produce an effect on energy metabolism, we first

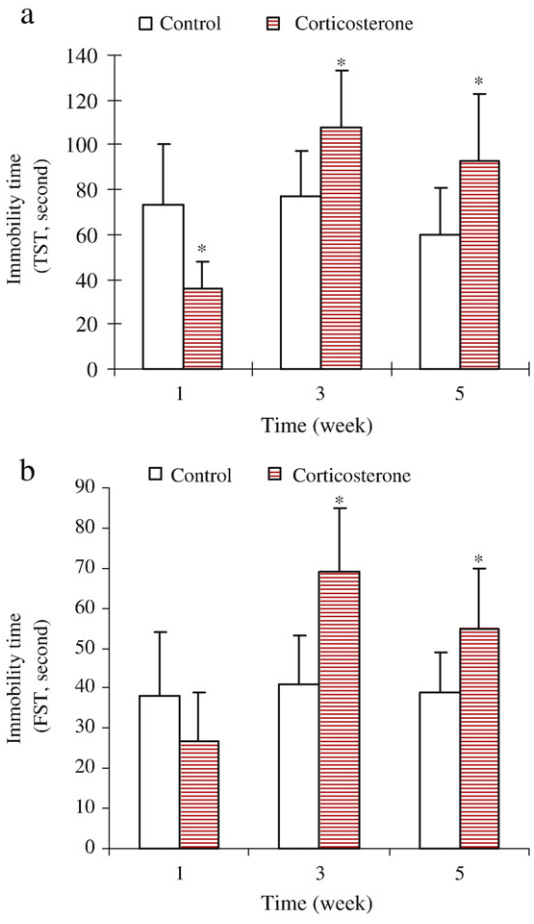

Fig. 1 – The results of the forced swim test and tail suspension test for the mice in each group ($n=15$): the mean (±S.D.) of time spent immobile during the last 4 min of the tail suspension test is shown in panel (a), the mean (±S.D.) of time spent immobile during the last 4 min of the swimming test is shown in panel (b). Data were analyzed by using two-way analysis of variance (ANOVA) with treatment and time as factors. *$P<0.05$, for the corticosterone vs. control mice, Tukey's Honestly Significant Difference test.