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Research Report

# Dehydroepiandrosterone protects male and female hippocampal neurons and neuroblastoma cells from glucose deprivation

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ABSTRACT

Dehydroepiandrosterone (DHEA) modulates neurogenesis, neuronal function, neuronal survival and metabolism, enhancing mitochondrial oxidative capacity. Glucose deprivation and hypometabolism have been implicated in the mechanisms that mediate neuronal damage in neurological disorders, and some studies have shown that these mechanisms are sexually dimorphic. It was also demonstrated that DHEA is able to attenuate the hypometabolism that is related to some neurodegenerative diseases, eliciting neuroprotective effects in different experimental models of neurodegeneration. The aim of this study was to evaluate the effect of DHEA on the viability of male and female hippocampal neurons and SH-SY5Y neuroblastoma cells exposed to glucose deprivation. It was observed that after 12 h of pre-treatment, DHEA was able to protect SH-SY5Y cells from glucose deprivation for 6 h (DHEA $10^{-12}$, $10^{-8}$ and $10^{-6}$ M) and 8 h (DHEA $10^{-8}$ M). In contrast, DHEA was not neuroprotective against glucose deprivation for 12 or 24 h. DHEA ($10^{-8}$ M) also protected SH-SY5Y cells when added together or even 1 h after the beginning of glucose deprivation (6 h). Furthermore, DHEA ($10^{-8}$ M) also protected primary neurons from both sexes against glucose deprivation. In summary, our findings indicate that DHEA is neuroprotective against glucose deprivation in human neuroblastoma cells and in male and female mouse hippocampal neurons. These results suggest that DHEA could be a promising candidate to be used in clinical studies aiming to reduce neuronal damage in people from both sexes.



## 1. Introduction

Steroid hormones are molecules mainly produced by endocrine glands, such as the adrenal glands, gonads and placenta, which are involved in the control of many physiological processes. In 1981, it was demonstrated that some steroids could be also produced by the nervous system, and the term “neurosteroids” was introduced to denominate the steroids that are synthetized in the nervous system independently of peripheral endocrine glands (Corpechot et al., 1981). Dehydroepiandrosterone (DHEA) was the first neurosteroid identified and is one of the most abundant circulating hormones in humans (Charalampopoulos et al., 2008a, 2008b; Corpechot et al., 1981). In addition to be classified as a neurosteroid, DHEA is also called neuroactive steroid, due to its modulatory actions on the central nervous system (CNS) (Stárka et al., 2015).

The physiological actions of DHEA involve both genomic and non-genomic mechanisms, including the activation of androgen/estrogen receptors and the allosteric modulation of multiple receptors (Hill et al., 2015). Previous studies revealed that DHEA modulates neurogenesis, neuronal function, neuronal survival and metabolism, enhancing mitochondrial oxidative capacity (Corpechot et al., 1981). DHEA and its sulfate ester (DHEAS) have been shown to attenuate excitotoxicity, protecting neuronal cells against excitatory amino acids and glucocorticoid toxicity (Kimonides et al., 1999; Kurata et al., 2004; Xilouri and Papazafiri, 2008). In addition, DHEA protects glial/neuronal mixed hippocampal cultures and human neuroblastoma cells against oxidative stress-induced damage (Bastianetto et al., 1999; Gao et al., 2005) and antagonizes the neurotoxic effects of corticosterone in primary cultures of hippocampal neurons (Kimonides et al., 1999). Moreover, DHEA and DHEAS protect against cell death induced by anoxia in rat embryonic cerebral cortical cultures (Marx et al., 2000).

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Furthermore, DHEA and DHEAS protect retinoic acid-differentiated SH-SY5Y cells from death induced by both staurosporine (a non-specific protein kinase inhibitor that is widely used for inducing mitochondrial apoptotic cell death) and doxorubicin (an activator of the death receptor signaling pathway) (Leoekiewicz et al., 2008). *In vivo*, DHEA and DHEAS are also neuroprotective against brain ischemia (Li et al., 2009). Additionally to its neuroprotective effects on brain ischemia *in vivo*, DHEA is also neuroprotective in neurotrophin-deprivation conditions (Lazaridis et al., 2011).

The brain is a highly oxidative organ that depends on a continuous glucose supply due to its limited capacity of energy stores (Dienel and Cruz, 2004). Glucose is essential to brain homeostasis and development (Lucas et al., 1988) and an inadequate glucose supply causes neuronal damage and increases the risk of neuropsychological dysfunctions (Almeida et al., 2002). Glucose deprivation, decreased blood supply and glucose hypometabolism have been also implicated in the neuronal death after brain ischemia and in Alzheimer's (AD) and Parkinson's disease (PD) (Albrecht et al., 2005; Baquer et al., 2009; Woo et al., 2010).

Hypometabolism in different regions of the brain, occurring as a consequence of multiple metabolic defects, is an early feature of AD (Moreira et al., 2009; Mosconi et al., 2008). Senile demented AD patients have reduced cerebral metabolic rate for glucose compared to elderly normal subjects and this decrease in glucose metabolism is correlated with cognitive performance, suggesting its use as a marker of disease progression (de Leon et al., 1983). AD patients present a reduced cerebral glucose transport activity (Kalaria et al., 1988), decreased levels of glucose transporters (GLUT1 and GLUT3) (Liu et al., 2008) and decreased glycolytic activity in their brains (Bigl et al., 2003). DHEA modulates metabolism, and the increase of glucose uptake can ameliorate brain metabolism of AD patients (Fuller et al., 2007). DHEA levels decrease with age and altered DHEA levels have been related to AD and dementia in humans (Genazzani et al., 2007; Weill-ENGerer et al., 2002). Previous work by our group showed an interaction between lactate and DHEA on the regulation of glucose uptake in different brain structures *in vitro* (De Souza et al., 2012). While DHEA had no significant effect on glucose uptake in the cerebellum, hippocampus or hypothalamus, it increased glucose uptake in the cerebral cortex in the presence of lactate and in the olfactory bulb in the absence of lactate, suggesting that DHEA may enhance glucose uptake in specific conditions (De Souza et al., 2012).

There are significant sex differences in the pathophysiology, epidemiology and clinical manifestations of many neurological diseases. Studies show that men are more susceptible to PD than women (Wooten et al., 2004) and present a higher mortality after stroke when affected before elderly (Reeves et al., 2008), possibly due to the lack neuroprotection provided by female steroids such as estrogen and progesterone. These observations are supported by studies showing that there are sex differences in the mechanisms that mediate neuronal death in different experimental models (Du et al., 2004; Lieb et al., 1995; Renolleau et al., 2008). Interestingly, it is known that male and female animals show different levels of neuroactive steroids, such as progesterone (PROG), tetrahydroprogesterone (THP), testosterone (T), dihydrotestosterone (DHT), DHEA and 3α-androstenediol (3α-diol) in some brain structures (Pesaresi et al., 2010), and this may contribute to the differences observed in the regulation of neuronal survival between both sexes. Nevertheless, up to date, few studies were performed to identify the effect of neuroactive steroids in the neuronal survival of cultures from different sexes. Therefore, the aim of this study was to evaluate the effect of DHEA on the viability of male and female hippocampal neurons and SH-SY5Y neuroblastoma cells exposed to glucose deprivation.

## 2. Results

### 2.1. Glucose deprivation reduced SH-SY5Y cell viability

In order to select a time of glucose deprivation that was able to induce cell death, we performed a time–viability curve. We analyzed the effect of 6, 8, 12 and 24 h of glucose deprivation in the viability of SH-SY5Y cells. Glucose deprivation reduced SH-SY5Y cell viability in all the times tested (Fig. 1).

### 2.2. DHEA protect SH-SY5Y cells from glucose deprivation

To determinate the optimal time and dose for DHEA treatment, cells were pretreated for 12 h with three doses of the hormone ($10^{-12}$, $10^{-8}$ or $10^{-6}$ M) and exposed to glucose deprivation for 6, 8, 12 and 24 h. DHEA was able to protect the cells from glucose deprivation for 6 h (DHEA $10^{-12}$, $10^{-8}$ and $10^{-6}$ M) (Fig. 2(a) and (b)) and 8 h (DHEA $10^{-8}$ M) (Fig. 2(c) and (d)). DHEA did not protect the cells against 12 h of glucose deprivation (Fig. 2(e) and (f)) or 24 h (Fig. 2(g) and (h)).

### 2.3. DHEA protects SH-SY5Y cells from glucose deprivation without pre-treatment

DHEA ($10^{-8}$ M) was able to protect SH-SY5Y cells from glucose deprivation for 6 h when the hormone was added at the beginning of glucose deprivation (i.e., no pre-treatment period) (Fig. 3) or when the hormone was added one hour after the beginning of glucose deprivation (Fig. 4).

### 2.4. DHEA protects male and female hippocampal neuronal cultures from glucose deprivation

After the identification of the neuroprotective effects of DHEA

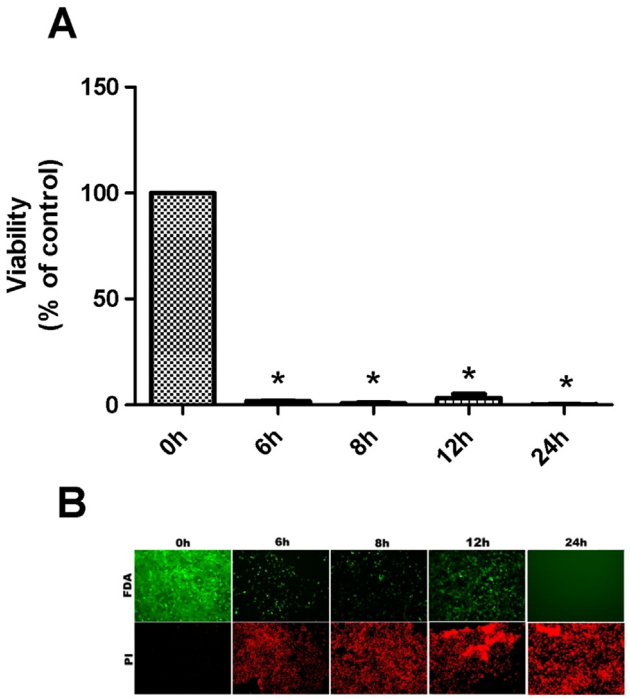


**Fig. 1.** Effect of glucose deprivation (6 h, 8 h, 12 h and 24 h) on SH-SY5Y cell viability. A: Ratio between the fluorescence emitted by alive/dead cells expressed as the percentage of control values. B: FDA-stained (alive) cells and PI-stained (dead) cells. Experiments were performed in triplicate. Results are expressed as mean ± SEM. N=6. *Different from control group ($P < 0.05$) (One-way ANOVA/Bonferroni).