# Dehydroepiandrosterone Protects Hippocampal Neurons Against Neurotoxin-Induced Cell Death: Mechanism of Action[2] (44437)

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*Abstract.* **Dehydroepiandrosterone (DHEA), an adrenal cortex hormone secreted in large quantities in humans, protects cells of the clonal mouse hippocampal cell line HT-22 against the excitatory amino acid glutamate (5 m*M*), and amyloid β-protein (2 μ*M*) toxicity in a dose-dependent manner with optimum protection obtained at 5 μ*M* concentration of DHEA. The protective effects of DHEA appear to be specific in that other related steroids and metabolites of DHEA, such as 5-androstene-3β,17β-diol, etiocholan-3α-ol-17-one, etiocholan-3β-ol-17-one, testosterone, and 5α-androstane-3,17-dione, offered no protection even at 50 μ*M* concentrations. In addition, using immunocytochemical techniques, we observed that 20 hr of treatment with 5 m*M* glutamate remarkably increased glucocorticoid receptor (GR) nuclear localization in neuronal cells. Interestingly, 5 μ*M* DHEA treatment for 24 hr, followed by 5 m*M* glutamate treatment for 20 hr almost completely reversed the copious nuclear localization of GR observed by glutamate treatment alone. Results obtained suggest that DHEA protects hippocampal neurons, at least in part, by its antiglucocorticoid action *via* decreasing hippocampal cells nuclear GR levels.** [P.S.E.B.M. 1999, Vol 222]

Dehydroepiandrosterone (DHEA) is an adrenal steroid secreted in large quantities in humans (30 mg/daily) and also synthesized in the human brain (1). For many years, the role of DHEA has focused on its place as an intermediate in sex steroid synthesis. More recently, DHEA has been shown to possess anticancer, antidiabetogenic, antiobesity and antiaging properties (2, 3). In addition, the antiglucocorticoid effects of DHEA have been observed by many investigators (3, 4). However, the precise physiological role and the mechanism of action of this hormone remains largely unknown.

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It is proposed that excitatory amino acids such as glutamate, as well as amyloid β-protein, are neurotoxic to cultured cells through their effects on antioxidant systems and a reduction in intracellular glutathione levels, leading to intracellular accumulation of peroxides and ultimately death (5). Interestingly, several groups have recently shown that estrogen protects neurons against glutamate and amyloid β-protein toxicity *in vitro* (6–8). These findings have generated considerable excitement in the scientific community and have raised suggestions that estrogen may not only be beneficial for the treatment of Alzheimer's disease but may also provide benefit to normal age-related memory loss (9). Since estrogen has been shown to have neuroprotective effects, in the present investigation we asked the question whether a neurosteroid, DHEA-like estrogen could provide protection against glutamate and amyloid β-protein-induced neurotoxicity. Since glucocorticoids are known to enhance oxidative stress–induced neuronal cell death (10), we have tested the hypothesis that DHEA's protective effects are mediated at least in part, through the modulation of glucocorticoid receptor.

## Materials and Methods

**Cell Culture and Chemicals.** The cell line HT-22 is a subclone of the HT4 hippocampal cell line. HT-22 cells

were a gift from Dr. David Schubert (The Salk Institute, San Diego California). The cell were cultured in DMEM supplemented with 10% FCS at 37°C, 10% $CO_2$. Amyloid $\beta_{25-35}$, monosodium glutamate, and all steroids were purchased from Sigma Chemical Co. (St. Louis, MO). All media, serum, and supplements were purchased from Gibco BRL (Grand Island, NY). All other chemicals used were of analytical grade.

**Cell Survival Assays.** Neuronal cell death was estimated using (i) microscopic examination of cells using phase contrast microscopy to evaluate morphological changes, and (ii) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

The MTT assays were performed in 96-well microtiter plates containing 100 μl of media per well. Following experimental treatments, 10 μl of a 5-mg/ml MTT stock in PBS was added to each well, and the incubation was continued for 4 hr. Finally, 100 μl of a solubilization solution containing 50% dimethylformamide, 20% SDS (pH 4.8) was added. The following day, absorption values at 570 nm were determined with an automatic microtiter reader. Phase contrast microscopy and cell counting were performed to assess morphological changes. All assays were performed in triplicate at least three times each.

**Hormone and Toxin Treatment.** Briefly, HT-22 cells (3000 cells/well) were plated in 96-well plates, and left untreated overnight. The medium was removed, and DMEM with 2% fetal calf serum was added. Hormones were added, and incubation proceeded for a period of 24 hr, followed by addition of toxin. After an additional 20 hr, cell viability was assessed by one of the previously described methods. Stock solutions of steroids ($10^{-2}$) were prepared in ethanol with a final ethanol concentration of 0.1%. Glutamate, and amyloid β-protein solutions were prepared in PBS.

*Immunocytochemistry.* Cells were plated on cover slips and treated with various experimental protocols. Slides were washed three times in PBS and fixed for 10 min at room temperature with 2% paraformaldehyde. The cells were washed three times in PBS and the permeabilized in PBS, containing 0.1% saponin and 0.25% gelatin, for 30 min and washed three times in PBS. The cells were then incubated for 30 min at room temperature in 0.2% normal goat serum followed by overnight incubation at 4°C with GR antibody diluted (1:200) in PBS. Cells were then treated for 30 min with biotinylated goat anti-rabbit IgG at a dilution of 1:200. Avidin-biotin peroxidase (1:200) was added, incubated for 30 min, and then treated for 10 min with diamino benzidine-hydrogen peroxide solution. Various controls, such as using nonspecific purified mouse IgM and IgG antibodies and preabsorbing GR antibody with partially purified GR receptor and preimmune rabbit serum (1:50 dilution), were used to assure glucocorticoid receptor specificity.

*Statistical Analysis.* Data were expressed as mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) and by Student's *t* test (two-tailed). A *P* value less than 0.05 was considered significant.

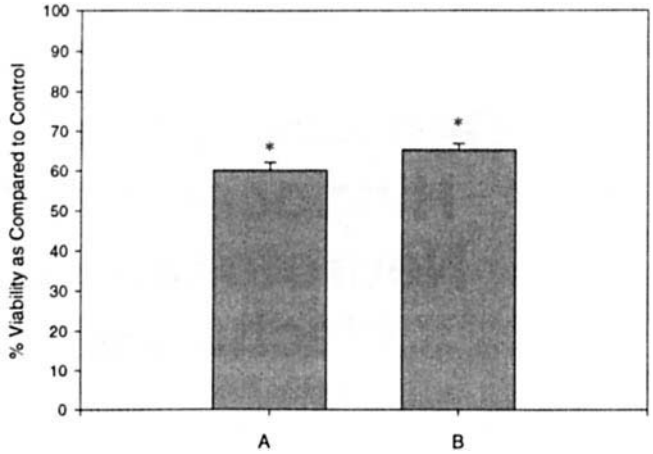


**Figure 1.** Glutamate or amyloid β-protein-induced neurotoxicity using HT-22 cells. Cells were treated with (A) 5 m*M* glutamate or (B) 2 μ*M* amyloid β-protein for 20 hr. Cell viability was determined using MTT assays as described in Methods. Percentage viability is expressed as 100% of control. Results presented are average of at least three experiments each done in triplicate and expressed as the mean ± SEM. *Significantly different from control levels ($P < 0.05$).

## Results

First, we determined the optimum dose of glutamate, or amyloid β-protein required for 40%–50% of HT-20 cell death as assessed by MTT assay. Data presented in Figure 1 showed that 5 m*M* glutamate, or 2 μ*M* amyloid β-protein was needed to obtain approximately 40% neuronal death of HT-22 cells. Therefore, these concentrations of neurotoxins were used for subsequent experiments.

Figure 2 represents the dose-response curve of DHEA

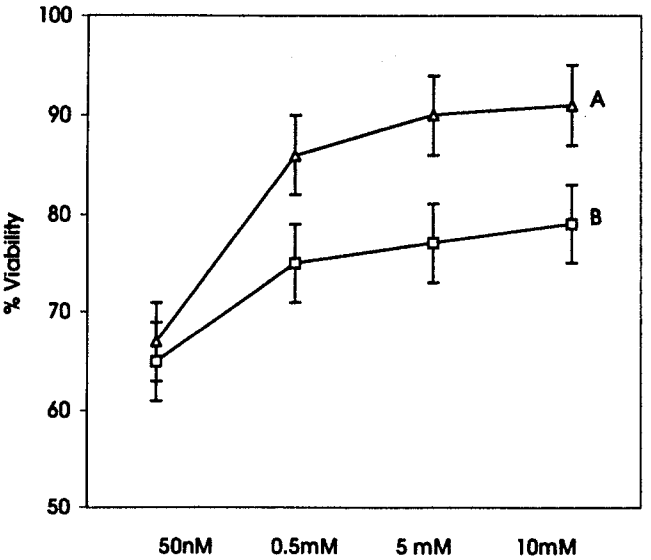


**Figure 2.** Dose response curve. HT-22 cells were treated with increasing concentrations of DHEA for 24 hr followed by 5 m*M* glutamate (A) or 2 μ*M* amyloid β-protein (B) for 20 hr. Cell viability was determined using MTT assays as described in Methods. Percentage viability is expressed as 100% of control. Results presented are average of at least three experiments each done in triplicate and expressed as the mean ± SEM.

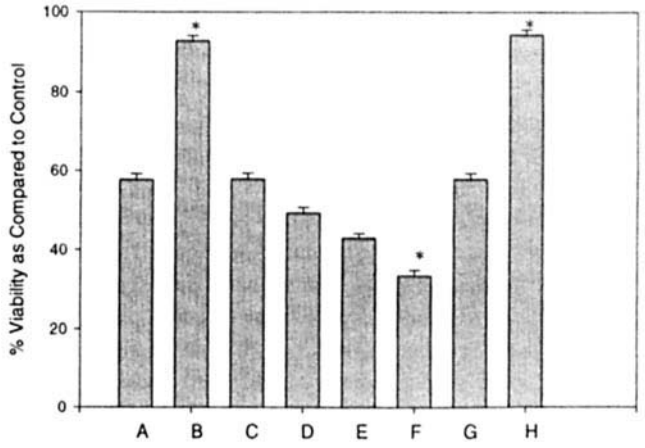


**Figure 3.** Steroid Specificity. HT-22 cells were treated with 5 μ*M* of indicated steroids for 24 hr followed by 5 m*M* glutamate for 20 hr. Cell viability was determined by MTT assays and expressed as 100% of control. (A) control, (B) DHEA, (C) 5-androstene 3β,17β-diol, (D) etiocholan-3α-ol-17-one, (E) etocholan-3β-ol-17-one, (F) testosterone, (G) 5α-androstane-3,17-dione, and (H) 17β estradiol. Results are average of three experiments done each time in triplicates and presented as the mean ± SEM. *Significantly different from control levels ($P < 0.05$).

protection against neuronal death induced by glutamate, or amyloid β-protein. DHEA protects HT-22 cells against the various neurotoxins tested in a dose-dependent manner, and optimum protection was observed at 5 μ*M* concentration of DHEA (Fig. 2).

Since DHEA is effective against both neurotoxins using HT-22 cells, we have used glutamate toxicity using HT-22 cells in all the subsequent experiments for simplicity.

Data presented in Figure 3 demonstrates the specificity of this observed effect. Several DHEA analogs were probed for their structure/function relationship in protecting against glutamate-induced neuronal death. Interestingly, 5 μ*M* of testosterone significantly induced cell death as compared to control, untreated cells. All other androgen-related steroids or DHEA metabolites, such as 5-androstene-3β,17β-diol; etiocholan-3α-ol-17-one; etiocholan-3β-ol-17-one; and 5α-androstane-3,17-dione, at 5 μ*M* concentrations were without any neuroprotective effect.

Following the glutamate challenge, phase contrast microscopy revealed significant changes in the general cellular morphology of hippocampal neurons evidenced by shortened dendrites and cell lysis (Fig. 4). Interestingly, DHEA completely prevented the morphological changes observed following glutamate toxic challenge (Fig. 4).

Immunocytochemical profiles of glucocorticoid receptors presented in Figure 5 revealed that control, untreated cells have less GR nuclear localization as judged from the intensity of immunostaining (Fig. 5A). Interestingly, neuronal cells treated for 20 hr with 5 m*M* glutamate revealed very high nuclear localization of GR (Fig. 5B), 5 μ*M* DHEA treatment for 24 hr, followed by 5 m*M* glutamate treatment for 20 hr remarkably suppressed the nuclear localization of GR (Fig. 5C).

## Discussion

The results presented here demonstrated that DHEA in a dose-dependent and steroid-specific manner protects HT-cells against glutamate, and amyloid β-protein-induced neuronal cell death. DHEA is shown to have a wide range of biological effects including its beneficial effects on cancer, obesity, immunity, and cardiovascular injury. However, results obtained demonstrating neuroprotective effects against various neurotoxins using neuronal cell lines are particularly interesting, as most biological effects of DHEA reported so far have used *in vivo* animal model systems, and hardly any information is available using *in vitro* cell culture systems (3,4). Kimonides *et al.* (11) recently reported that 100 n*M* DHEA protects rat fetal hippocampal cells against 1 m*M* N-methyl-D-aspartic acid (NMDA) or 1 m*M* kainic acid–induced neurotoxicity. Unlike their results, we needed at least a 5-μ*M* concentration of DHEA to protect HT-22 cells against both glutamate or amyloid β-protein toxicity. It is possible that fetal neuronal cells are more susceptible to neurotoxin and may require smaller concentrations of DHEA than HT-22 cells.

Despite its wide range of biological effects, the cellular and molecular mechanisms of action for DHEA remains

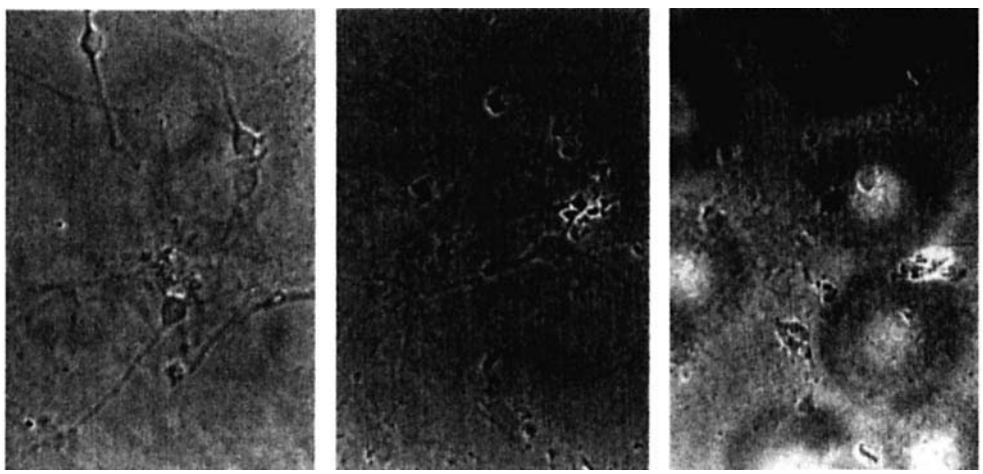


**Figure 4.** Morphological profile of glutamate-induced toxicity and neuroprotection by dehydroepiandrosterone. HT-22 cells were treated for 20 hr with (A) control, (B) 5 m*M* glutamate, or (C) 5 μ*M* dehydroepiandrosterone followed by 5 m*M* glutamate for 20 hr. Cells were examined by phase contrast microscopy as described in Methods.

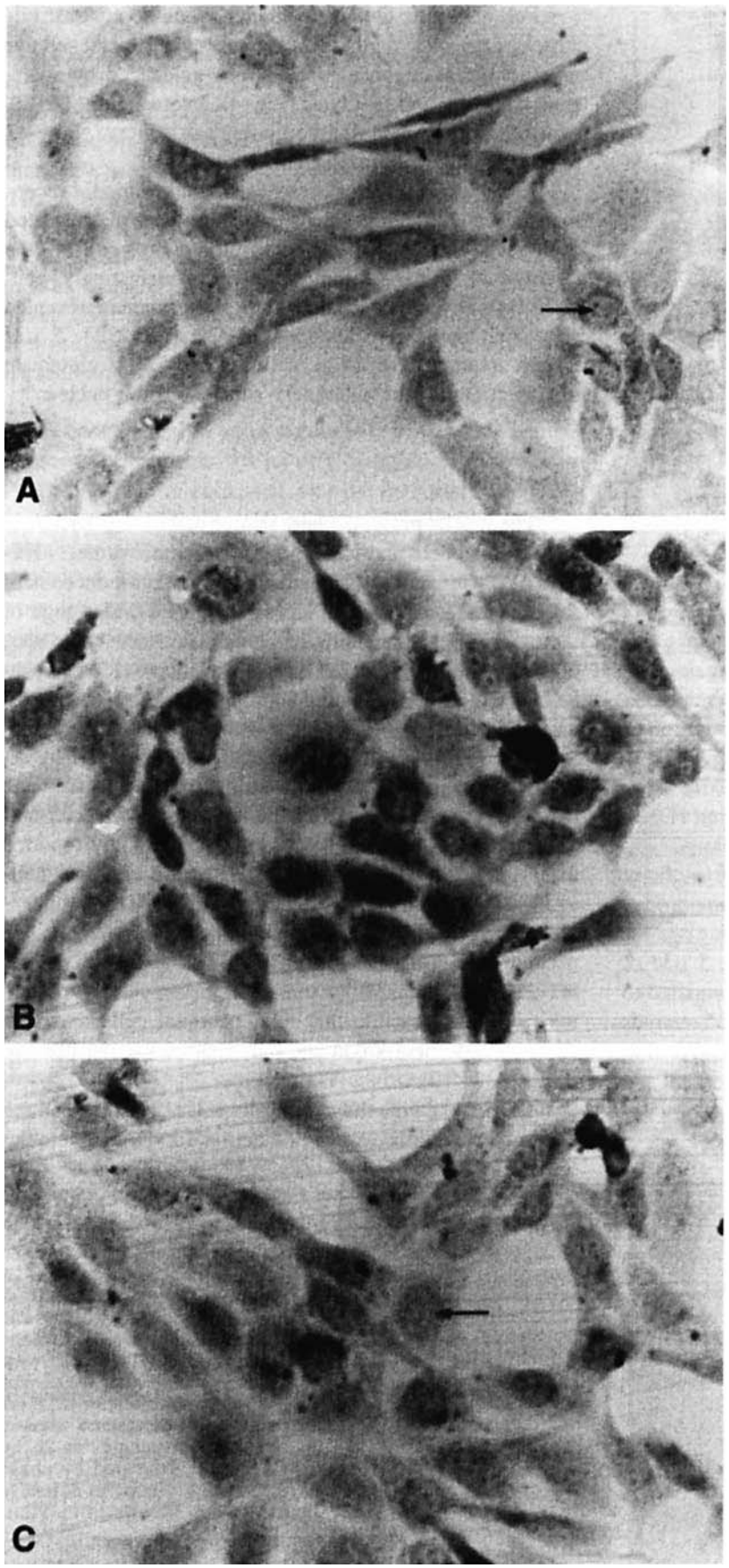


**Figure 5.** Immunocytochemical localization of GR in HT-22 cells. The cells were fixed and processed as described in Methods. (A) control, (B) 5 m*M* glutamate, and (C) 5 μ*M* dehydroepiandrosterone followed by 5 m*M* glutamate.

largely unknown. It is possible that DHEA protects against neurotoxicity by binding directly to its own specific receptor, or it possibly can mediate its effects through regulation of the GR (3, 4). Our data using immunocytochemical techniques suggest that DHEA modulates cellular GR nuclear localization and exerts its neuroprotective effects against glutamate toxicity by lowering GR nuclear localization. The hippocampus is crucial in our responsiveness and adaptation to stress-related HPA-axis and is highly enriched in GR. Since glucocorticoids are known to increase hippocampal neuronal cell death (10) and DHEA protects against it, it is fair to postulate that the neuroprotective effects of DHEA are mediated, at least in part, through its antiglucocorticoid effects. Despite this novel modulation of nuclear GR by DHEA, we cannot rule out the possibility that besides GR-inhibiting effects, DHEA may modulate various neurotransmitters directly and/or their receptors.

In conclusion, the hippocampus is known to be highly vulnerable to neuronal degradation in the brain pertinent to Alzheimer's disease (AD) and other age-related neurological disorders. Therefore, our findings on the neuroprotective effects of DHEA may have physiologic or pharmacologic relevance. This might have clinical implications in the treatment or prevention of various neurodegenerative diseases. In addition, the neuroprotective effects of DHEA action that we observed, using HT-22 cells, may provide a good *in vitro* model to achieve an understanding of the various signaling pathways involved in the cellular and molecular action of DHEA.


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