Research report

Dehydroepiandrosterone (DHEA) reduces neuronal injury in a rat model of global cerebral ischemia

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Abstract

Introduction: Many studies report an inverse correlation between levels of DHEA and neurological diseases. Exogenous DHEA protects hippocampal neurons against excitatory amino acid induced neurotoxicity. The purpose of this experiment is to evaluate the effect of DHEA in an animal model of transient but severe forebrain ischemia. Methods: At thirteen days prior to induction of ischemia, male Wistar rats were implanted with various doses of DHEA–placebo, 25 mg, 50 mg or 100 mg. Forebrain ischemia was induced for 10 min using a modified four-vessel occlusion technique, with hippocampal neuronal injury assessed at 7 days post-ischemically and expressed as a percentage of total cells. Results: Both normal and necrotic hippocampal CA1 cells were counted. Percentages of hippocampal injury observed were 88±13% in animals treated with placebo, 84±8% in the 25 mg DHEA group, and 60±7% in the 50 mg DHEA group. Animals treated with 100 mg DHEA displayed a significant (P<0.05) reduction of hippocampal CA1 cell injury at 60±7%. Conclusion: Treatment with a high dose, but not a low or moderate dose, of DHEA implantation reduces hippocampal CA1 neuronal injury following severe but transient forebrain ischemia.

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1. Introduction

Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) are the major secretory products of the adrenal gland. The serum concentration of DHEAS is between 300 and 500 times higher than that of DHEA, although DHEA is probably more active at the tissue level. Brain de novo can generate DHEA (termed neurosteroid), and its concentration is higher than that in the periphery [17,6]. Decreases of DHEA have been linked to the development of several neurological diseases [4,6].

Transient forebrain ischemia causes irreversible neuronal degeneration to highly sensitive regions of the brain. In particular, the hippocampal CA1 cell is exquisitely vulnerable to delayed injury following ischemia [15,21]. Release of glutamate and subsequent calcium entry contribute to ischemic CA1 neuronal cell injury [7]. Blocking the effects of glutamate using NMDA receptor [13] or AMPA receptor [13] antagonists can reduce neuronal injury.

Various studies show that DHEA(S) possess multiple biological activities, such as neurotrophic effects [23,12], modulation of NMDA receptor and calcium channel [6], up-regulation of neuronal excitability [18], and blocking effects of glucocorticoid [3]. Both in vivo and in vitro studies have demonstrated that treatment with DHEA can reduce NMDA-induced neurotoxic effects [14] and oxidative stress-induced damage [5] in the hippocampal CA1 region.

Currently there are no animal experimental data available to support the roles of DHEA and its sulfate (DHEAS) in cerebral ischemia. The purpose of this experiment was to determine whether DHEA offered neuroprotection in a rat model of transient but severe forebrain ischemia.
2. Material and methods

Male Wistar rats, weighing 150–175 g (Charles River, Montreal, Canada), were used in this study. Pellets of DHEA or placebo, purchased from Innovative Research of America (Sarasota, FL, USA), were implanted dorsally in the subcutaneous tissue of the neck 12 days before surgical preparation for ischemia, and remained in the body until the animals were sacrificed. Animals were placed into one of the following four groups: placebo (n=9), 25 mg DHEA (n=6), 50 mg DHEA (n=9), or 100 mg DHEA (n=8).

The surgical method for preparation of 4-vessel occlusion (4-VO) has been previously described in detail [22]. In brief, the animals were anesthetized with a mixture of 2% halothane, 70% N2, and 28% O2. Reversible ligatures were placed around both common carotid arteries and both vertebral arteries were cauterized. The wounds were closed and animals were returned to their cages overnight.

The following day, the animals were subjected to 10 min of forebrain ischemia by tightening the ligatures around the carotid arteries and the para-vertebral muscles in which the collateral arteries are located. Body temperature was maintained at 37.5°C. Seven days following ischemia the animals were reanesthetized and perfusion-fixed with a transcardiac infusion of heparinized saline, followed by 4% buffered formalin. Serial 7 μm sections were cut and stained with hematoxylin and eosin. The number of necrotic and normal cells in the CA1 region of the hippocampus were counted and expressed as mean±standard deviation of percent neuronal injury. The percentages were compared with the Kruskal–Wallis test and the Mann–Whitney U test with Bonferroni corrections. This sample size, through a priori calculation using an expected mean of 80±20%, was adequate to detect a 30% difference among groups (α=0.05, β=0.2). All data are expressed in percentage mean±S.D.

3. Results

3.1. Mortality

Two animals from group 2 (DHEA, 25 mg) and one animal from group 4 (DHEA, 100 mg) died during the ischemic period and were thus excluded from the study. Animals exhibited normal weight gain and did not show any abnormal behaviour. All animals maintained a body temperature of 37.5°C following the DHEA implantation and ischemia. All animals met ischemic criteria and DHEA did not influence the behaviour of the animals during or following 4-VO.

3.2. Neuropathology

Histological outcomes of individual animals are presented in Fig. 1. Hippocampal CA1 cell injuries were not significantly different among placebo (88±13%), DHEA 25 mg (84±8%), and DHEA 50 mg (82±6%) groups. In the DHEA higher dosage group (100 mg), a mild but significant reduction of neuronal injury in the hippocampal CA1 region was measured. In this group, neuronal injury was reduced to 60±7% from 88±13% seen in the placebo group (P<0.05).

4. Discussion

This is the first animal experiment to test the efficacy of DHEA as a neuroprotectant in cerebral ischemia. The results from this experiment demonstrate that a high dose, but not a low or moderate dose, of DHEA reduces neuronal injury following transient and severe forebrain ischemia. Although the neuroprotection is mild and weak, DHEA might be used as supplementary neuroprotective agent for future stroke treatment.

The molecular basis of selective CA1 neuronal injury following ischemia remains speculative. Excessive glutamate release and resulting calcium entry, as well as increased post-ischemic sensitivity to excitatory neurotransmitters, may be key factors in CA1 neuronal loss [7,25,9]. Antagonists of the amino-T-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors have been found to reduce CA1 loss [16,24]. SNX-111, which blocks N-type Ca2+ channels and prevents glutamate release, has been shown to reduce CA1 injury [25,9]. Moreover, a previous study suggests that reducing the inflammation incurred by ischemia can also reduce CA1 cell injury [19]. Treatment with DHEA has been shown to protect
primary hippocampal culture against NMDA induced toxicity [14] and enhance survival rates of cultured neurons [8]. In vivo, subcutaneous pellets of DHEA protected hippocampal CA1 neurons against unilateral infusions of NMDA [14]. We have used the same pellet method of DHEA administration and therefore infer that our plasma levels were similar to Kimonides et al., who measured DHEA to be protective at 16.5 ± 1.8 ng/ml. The results of Kimonides et al. were felt to suggest that a decreased DHEA level may contribute in part to the increased vulnerability of the aging or stressed human brain to such damage [2].

In the current experiment, the mild reduction of ischemic neuronal injury demonstrated with treatment by DHEA might suggest that NMDA is not a main contributor in the degenerative process involving hippocampal CA1 cells following cerebral ischemia. Neuroprotective effects by NMDA glutamate antagonists have not been universally supported in animal studies. Experimental data has demonstrated that NMDA antagonists (MK-801) were unable to prevent selective neuronal death following brief but severe forebrain ischemia [10,20], although they might reduce cortical infarction in focal ischemia [11]. This is possibly as a result of altered regional cerebral blood flow, which is possible in focal but not global ischemia. In this experiment, which used transient but severe forebrain ischemia, blood flow is severely attenuated and not easily influenced. The behaviour of the DHEA-treated animals during ischemia did not suggest any improvement in flow that might account for possible neuroprotective effects.

In a clinical setting, this may be a more complex issue. In men, DHEA acts like an estrogen and may protect against cardiovascular disease [1]. In post-menopausal women, metabolism of DHEA to testosterone may actually occur [14] and may explain why the estrogen-like effects of DHEA are protective in men, while the opposite may be true in post-menopausal women.

DHEA is now widely available from a variety of sources. It has been extensively promoted as an ‘alternative’ therapy that prevents the effects of aging, boosts immunity to cardiovascular disease, offers natural weight control, prevents cancer, enhances brain function, and extends life.

References


