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Antiepileptic action of exogenous dehydroepiandrosterone in iron-induced epilepsy in rat brain

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ABSTRACT

In the study described here, the antiepileptic effect of dehydroepiandrosterone (DHEA) treatment on iron-induced focal epileptiform activity in the rat brain was investigated. DHEA is a neuroactive corticosteroid hormone synthesized both in the adrenal cortex and in the brain. Its antioxidant properties are well known. As oxidative stress seems to play a major role in epileptogenesis in the iron-induced model of posttraumatic epilepsy, it was of interest to examine whether DHEA would exert antiepileptic activity. DHEA at a dose of 30 mg/kg/day administered intraperitoneally for 7, 14, and 21 days to iron-induced epileptic rats prevented epileptiform electrophysiological activity. Morris water maze and open-field tests on iron-induced epileptic rats revealed that DHEA also prevented behavioral alterations related to epileptiform activity. Thus, DHEA attenuated the cognitive defects produced by epileptic activity. Moreover, alterations in epileptogenesis-related biochemical parameters—lipid peroxidation, protein oxidation and Na^+, K^+-ATPase (sodium pump) activity—were also countered by DHEA.

1. Introduction

Iron-induced (experimental) epilepsy as a model of human posttraumatic epilepsy (PTE) was first proposed by Willmore et al. [1]. Development of PTE in humans follows closed-head injury in many cases. Posttraumatic epileptic activity is thought to be caused by the iron released from extravasated hemoglobin. Iron–neuronal tissue interaction mediated by iron-induced oxidative stress appears to make the neurons epileptogenic. Experimentally, a single injection of several microliters of ferrous/ferric chloride into rat or cat somatosensory cortex by stereotaxic procedures produces a chronic epileptogenic focus. This experimental model has often been used to investigate the mechanism of epileptogenesis as well as the pharmacology of epilepsy [2–6]. This model was adopted in the present study to investigate the possible antiepileptic effect of dehydroepiandrosterone on iron-induced focal epileptiform activity in rat brain.

Dehydroepiandrosterone (DHEA, 3α-hydroxy-5-androstene-17-one) is a natural physiological hormonal substance synthesized from cholesterol in the central nervous system of adult rats, in the hippocampus [7,8] as well as in the adrenal cortex. DHEA is a multifunctional steroid in the central nervous system [9]. The brain is also regarded as a target for steroid hormones synthesized by the adrenal glands [10]. The level of DHEA declines with age and during certain types of illnesses or stress. DHEA possesses antioxidant properties [11,12], and it has been shown to be neuroprotective against oxidative stress in rat hippocampal neuronal cultures [13] and hippocampal damage induced by *N*-methyl-D-aspartate (NMDA) receptor excitotoxicity [14]. A decrease in DHEA sulfate levels was measured in women with high-frequency seizures [15]. Therefore, a decrease in DHEA levels may contribute significantly to the increased vulnerability of the aged or stressed human brain to dysfunctions, disorders, or diseases [14,16]. DHEA also prevents many age-dependent morphological, physiological, and behavioral alterations in the central nervous system and has, therefore, been considered to be a neuroactive pharmacological substance [16,17]. It influences neuronal activity via interaction with neurotransmitter receptors including NMDA and σ- and γ-aminobutyric acid (GABA) receptors [18], although the exact role of each of these neurotransmitter receptors with respect to DHEA is not well established. Beneficial effects of DHEA have been reported in several clinical studies [19,20].

The objective of the current experiments was to determine whether DHEA has an antiepileptic effect. It is known that sex hormones (such as progesterone and estrogen) exert significant influence on the epileptogenetic process [21]. In an animal model of atypical absence seizures, estrogen is an anti-absence seizure hormone, whereas progesterone is a pro-absence seizure hormone, and hormones are also involved in the control of neuronal excitability

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[21,22]. There is an increased incidence of epilepsy in the elderly human population, indicating that the aged central nervous system may become more susceptible to epileptogenesis [23]. Glutamate receptors tend to become more sensitive during aging. DHEA, in addition to being an anti-lipid peroxidative agent, has complex effects on GABA/glutamate receptors that are involved in the control of neuronal excitability [17]. Furthermore, recent studies have emphasized the impact of neuroactive steroids on the physiology and pharmacology of epileptic disorders [10,24]. In view of the above findings, it was of interest to examine whether pharmacologically DHEA would also have antiepileptic effects.

Therefore, in the present study, we studied the effects of DHEA on epileptic seizure activity in iron-induced chronic epileptogenic foci in rat brain. Antiepileptic influence was ascertained on the basis of epileptiform electrophysiological activity, biochemical parameters (lipid peroxidation, protein oxidation, Na^+, K^+-ATPase activity), and cognitive-behavioral parameters as assessed with the Morris water maze and open-field tests.

2. Material and methods

2.1. Materials

All stainless-steel electrodes and wires used in electrophysiological surgery and recordings were tissue compatible and obtained from Plastic One (Roanoke, VA, USA). DHEA and other chemicals were obtained from Sigma Aldrich Chemical Company USA. All chemicals used were of analytical grade.

2.2. Animals and treatment

Twenty-five male Wistar rats 8–10 months of age and weighing 400–450 g were used for this study. Animals were housed in pairs in standard $8\times12\times5$-in. laboratory cages made of polypropylene with stainless-steel covers, and maintained at 23 ± 4 °C, under a 12-hour-light/12-hour-dark cycle. All experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) and the Institutional Animal Ethical Committee of Jawaharlal Nehru University, New Delhi, India. Each animal was provided ad libitum access to food and water. The health status of each rat was checked by observing various criteria such as tail sores, posture hunch, grooming, nose red rim, red eye rims, tumors, and teeth.

Dehydroepiandrosterone was dissolved in 0.1% dimethyl sulfoxide (DMSO) and administered to rats at the dose of 30 mg/kg body wt in accordance with the procedures described by Wen et al [25]. DMSO has been used to dissolve neurosteroids [26], and at various dose levels it does not affect neuronal electrical firing [27] and also has been found not to alter membrane potential and ionic currents [28]. In a pilot experiment, we found that DMSO (vehicle) given alone for up to 21 days at the 0.1% dose level does not alter epileptiform electrographic and seizure activity. DHEA treatment was started on day 20 after intracortical iron injection.

Animals were grouped as follows. Group I ($n=5$) consisted of sham controls (these animals received intracortical injections of saline); group II ($n=5$) consisted of iron-induced epileptic rats (rats were made epileptic by intracortical injection of ferric chloride using the procedure described below). In these rats, development of epileptiform electroencephalographic activity was studied. These rats were treated with vehicle (DMSO) and served as controls for the next three groups of DHEA-treated rats. Treatment with vehicle began on day 20 after iron injection; group III ($n=5$) consisted of iron-induced epileptic rats that received one intraperitoneal injection of DHEA per day for 7 days; group IV ($n=5$) consisted of epileptic rats that received DHEA for 14 days; and group V ($n=5$) consisted of rats that received DHEA treatment for 21 days.

2.3. Surgical procedure and recordings

Surgical procedures were performed under ketamine (80 mg/kg)- and xylazine (10 mg/kg)-induced anesthesia. Burr holes 0.5 mm in diameter (one for iron injection and four for placement of epidural electrodes, two on the left and two on the right) were drilled into the skull bone at stereotaxically marked sites. $FeCl_3$ (5 μL containing 100 mM $FeCl_3$ dissolved in physiological saline) [1] was stereotaxically injected over 5 minutes through the burr hole (coordinates: 1.0 mm anteroposterior, 1.0 mm lateral, and 1.5 mm ventral from bregma) in the somatosensory region of the cortex with the help of an injector cannula. After injection, the burr hole was sealed with bone wax. For recordings from the hippocampus, intracerebral bipolar electrodes were stereotaxically placed in the CA1 region (coordinates: 5.3 mm posterior and 3 mm lateral from bregma and 3 mm ventral to dura) and in the CA3 region (3.8 mm posterior and 4 mm lateral from bregma and 5.5 mm ventral to dura) according to the atlas of Paxinos and Watson [29]. Coordinates for four epidural cortical (stainless-steel screws) electrodes were: 2 mm posterior and anterior to bregma and 2 mm lateral to the midline. One screw electrode was also placed on the frontal sinus to serve as animal ground. The free ends of these electrodes were soldered to a nine-pin connector, which was fixed to the skull with dental acrylic to make a robust platform. Operated rats were provided with optimal postoperative care and habituation in the recording chamber for 5 days before electrophysiological recordings were obtained using a Grass EEG/ polygraph recorder (Model 79D).

For multiple-unit activity (MUA) recordings, composite extracellular signals from the electroencephalographic electrodes were routed through a high-impedance probe (Grass HIP511), amplified and filtered (300 Hz to 10 kHz) by Grass P511 preamplifiers, electronically discriminated, and displayed on an oscilloscope. The standard output pulses from the window discriminator (WPI) were also simultaneously recorded on the polygraph. The recordings were limited to the awake immobile state in which a rat sits quietly but remains awake as described in our previous studies [2,4,30,31].

2.4. Preparation of homogenate

Rats were killed by cervical dislocation after electrophysiological and behavioral recordings. Brains were quickly removed and cooled in a deep freezer. Cortex and hippocampus were rapidly dissected out on an ice plate. The left and right hippocampi of the brain of one rat were pooled to make one tissue sample. Biochemical assays were performed separately in five animals of each group. Tissue samples were homogenized in the 50 mM Tris (pH 7.4)-containing Protease Arrest Kit from Geno Technology Inc. (St. Louis, MO, USA) in Potter–Elevehjem-type homogenizer fitted with a Teflon plunger. The homogenate was diluted 1:10 with Tris (pH 7.4) buffer and centrifuged at 6000 rpm for 10 minutes in a refrigerated centrifuge (Sorvall RCS or RC5C). The resulting pellet, consisting of nuclear and cellular material, was discarded. The supernatant was further centrifuged at 14,000 rpm for 25 minutes to separate synaptosomes and mitochondria from microsomes and cytosol [32]. Na^+, K^+-ATPase activity was measured in the crude synaptosomal fraction, whereas lipid peroxidation and protein oxidation were measured in the cytosolic fraction.

2.5. Biochemical assays

Protein carbonyl content, as an index of protein oxidation, was measured with the modified method described by Liu et al. [33]. For each homogenate, a sample containing 2 mg of soluble proteins was divided into two equal volumes. Four volumes of 10 mM dinitrophenol hydrazine (DNPH) in 2 M HCl was added to one of the sample pair, and 4 vol of 2 M HCl alone was added to the other. The samples were then incubated for 1 hour at room temperature in the dark with