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Dehydroepiandrosterone as an inducer of mitochondrial permeability transition

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Abstract

This paper reports an investigation upon the effect of dehydroepiandrosterone (DHEA) on some mitochondrial membrane functions, such as electron transport, transmembrane electric gradient and calcium permeability. It was found that the hormone induced the efflux of accumulated matrix Ca^{2+}, inhibited Site I of the respiratory chain, as well as bringing about the collapse of the transmembrane potential, and mitochondrial swelling. Taking into account that cyclosporin A (CSA) inhibited Ca^{2+} release and the collapse of the transmembrane potential, it is concluded that the hormone may induce the opening of a non-specific transmembrane pore. The mechanism of pore opening is ascribed to peroxidation of the membrane lipid bilayer. It should be mentioned that estrone, even at the concentration of 200 μM, failed to reproduce the behavior of dehydroepiandrosterone on mitochondrial functions.

Keywords: Dehydroepiandrosterone; Kidney mitochondria; Permeability transition; Calcium; Cyclosporin A

1. Introduction

Dehydroepiandrosterone (DHEA), a precursor of male and female sex hormones, has been used with apparent beneficial effects in aging, Alzheimer's disease and dementia [1,2]. Furthermore, it has been discussed that DHEA improves cognitive functions in normal old adults [3]. However, the beneficial effects are questionable because it has been found that the steroid induces serious deleterious side effects, i.e., apoptosis, hepatic carcinoma, and acts as inhibitor of cellular proliferation [4,5]. It should be noted that DHEA therapy requires, in some instances, high doses, i.e., 50–100 mg/day [3,6]. At subcellular level Mohan and Cleary [7,8] provided evidence that the addition in vivo and in vitro, of DHEA inhibits mitochondrial respiration supported by NAD-dependent substrates; however, it does not inhibit succinate oxidation. Zheng and Ramírez [9] demonstrated that DHEA inhibits F_0F_1-ATPase activity. Swierczynski and Mayer [10] found that in vivo administration of DHEA to rats induces lipid peroxidation in liver, kidney, and heart mitochondria. In this respect, it has been demonstrated that peroxidation of mitochondrial membrane lipids promotes an increased permeability [11–13]. This process, known as permeability transition, is characterized by the opening of a non-specific transmembrane pore that allows the efflux of matrix contents with a molecular mass <1500 Da [14]. Considering the effect of DHEA on membrane lipids, this work was undertaken with the purpose to explore the possibility that such steroid may induce membrane leakage. The results of this study show that, in isolated rat kidney mitochondria, DHEA induced a fast release of accumulated matrix Ca^{2+}, and inhibited the oxidation of malate–glutamate by blocking the electron transport in Site I of the respiratory chain. In addition, it was found that DHEA induced mitochondrial swelling, and collapse of the transmembrane electric gradient ($\Delta\Psi$). These effects were suppressed by cyclosporin A (CSA), and reversed by bovine serum albumin (BSA). Succinate partially reversed Ca^{2+} efflux and drop of $\Delta\Psi$. Interestingly, estrone even at the concentration 200 μM did not affect mitochondrial functions. Our proposal is that DHEA opens the non-specific transmembrane pore through promotion of lipid peroxidation.

2. Materials and methods

Kidney cortex mitochondria were prepared after homogenization of the tissue in 0.25 M sucrose–1 mM EDTA, adjusted to pH 7.3, following the standard centrifugation procedure. The last washing was carried out in 0.25 M

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sucrose without EDTA. Submitochondrial particles (SMP) were prepared by sonication of mitochondria during three sessions of 1 min each; after centrifugation at 17,000 × *g*, the supernatant was spun down at 100,000 × *g* during 60 min. The pellet was suspended in EDTA-free sucrose. Protein was determined by the method of Lowry et al. [15]. Mitochondrial Ca^{2+} movement was followed spectrophotometrically at 675–685 nm, using the metallochromic indicator Arsenazo III as reported by Scarpa et al. [16]. Transmembrane potential was analyzed by measuring the changes in absorbency at 511–533 according to Akerman and Wikström [17], using Safranine as monitor. Mitochondrial swelling was followed by changes in optical density at 540 nm. Oxygen consumption was measured polarographycally by using a Clark type electrode coupled to a recorder. The basic medium contained 250 mM sucrose, 5 mM malate, 5 mM glutamate, 3 mM phosphate, and 2.5 μg oligomycin. Other additions were as indicated in the respective legends of the figures. Lipid peroxidation was estimated by measuring the generation of thiobarbituric acid-reactive substances as reported [18]. For the in vivo experiments, and in agreement with Berdanier and McIntosh [19] the rats received 120 mg/kg DHEA i.p. for 2 days with 120 mg/kg, instead of 4 weeks as indicated these authors.

3. Results

Mohan and Cleary [7,8] have demonstrated that DHEA inhibits the oxidation of NAD-dependent substrates. Since rotenone, an inhibitor of the electron transport at Site I, induces membrane permeability transition [20], we explored the possibility that the hormone may induce the opening of the non-specific pore. Fig. 1A shows that indeed, in mitochondria oxidizing malate/glutamate, increasing concentrations of DHEA, from 100 to 233 μM induced the release of accumulated Ca^{2+}. Considering the high concentrations of DHEA used the question arises on the possibility that other steroid may promote permeability transition, in an unspecific fashion. Fig. 1B illustrates that estrone, whose structure resembles, to some degree, that of DHEA, was unable to discharge accumulated Ca^{2+}, even at the concentration of 200 μM.

To ascertain if under our conditions DHEA blocks the electron transport in Site I, the following experiments were performed. Fig. 2A shows that the addition of 166 μM DHEA to mitochondria, oxidizing malate/glutamate, causes an inhibition by approximately 82% of the ADP-stimulated respiratory rate, i.e., from 44 to 8 nAO/min/mg. It is also shown that the inhibition was totally reversed when

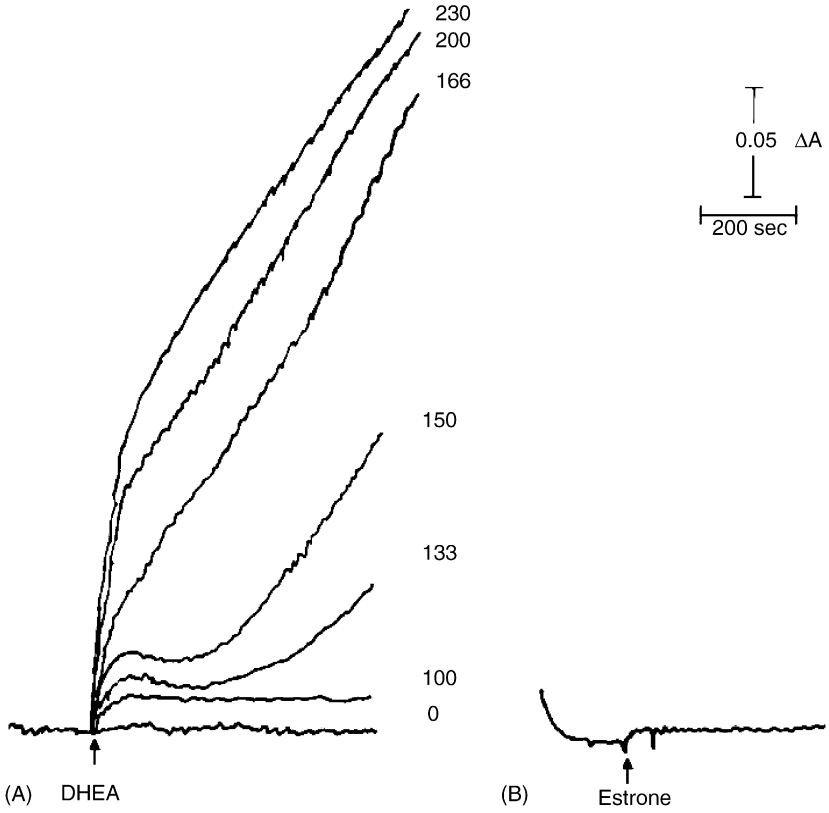

Fig. 1. The effect of increasing concentrations of DHEA, as well as the effect of estrone, on mitochondrial Ca^{2+} efflux. Two milligram of mitochondrial protein were added to 3 ml of the basic medium described under Section 2. In addition, the medium contained 50 μM $CaCl_2$, and 50 μM Arsenazo III. In Panel A, the numbers at the side of the traces signify micromolar concentrations of DHEA. Where indicated in Panel B 200 μM estrone was added. Temperature, 22 °C.

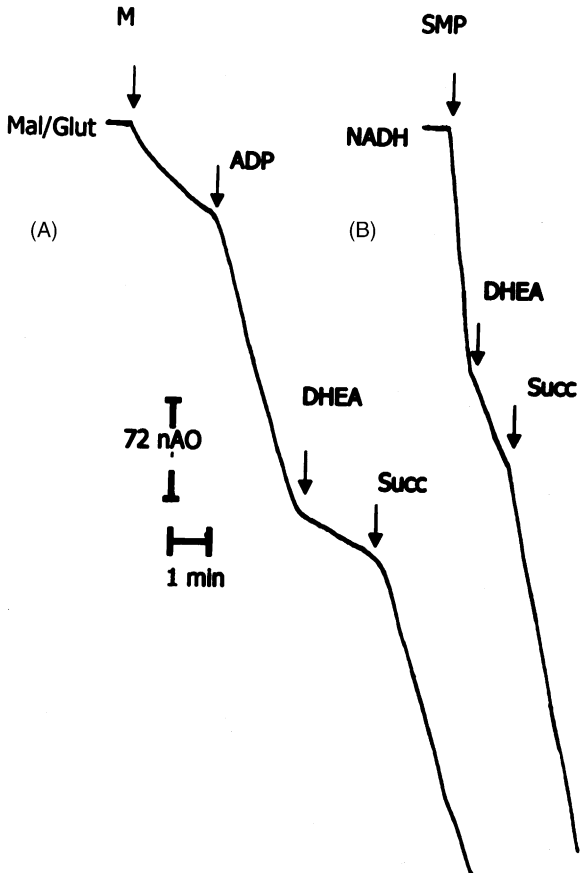

Fig. 2. The effect of DHEA on oxygen consumption. The 2 mg of mitochondria and 2 mg of submitochondrial particles (SMP) were added in (A) and (B), respectively, to 1.7 ml of basic incubation mixture described under Section 2. Where indicated 3.5 mM ADP, 166 μM DHEA, 0.05% BSA, 5 mM succinate or 5 mM NADH were added. Temperature, 22 °C.

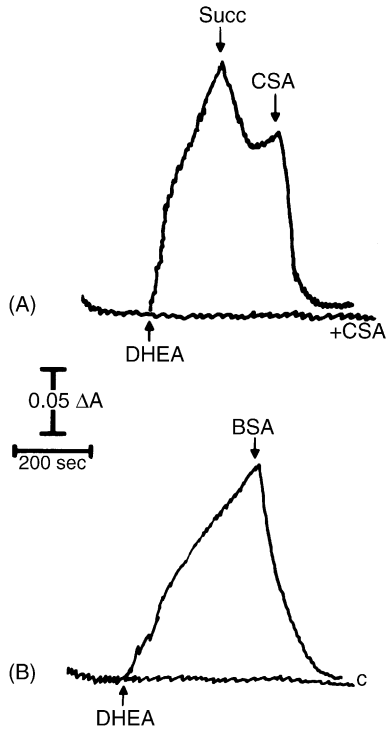

Fig. 3. Effect of CSA, BSA, and succinate on DHEA-induced Ca^{2+} efflux. Experimental conditions as referred in Fig. 1. Where indicated 166 μM DHEA, 0.5 μM CSA, 5 mM succinate, or 0.05% BSA were added.

succinate was added to the incubation mixture: from 8 to 45 nAO/min/mg. With the aim to discard a possible inhibition on the transport of malate and glutamate or an inhibition on the respective dehydrogenases, the effect of DHEA was analyzed in submitochondrial particles. Fig. 2B shows that DHEA inhibited NADH oxidation by a similar percentage that it does in whole mitochondria. This result points to Site I of the respiratory chain as the target for DHEA, and discards an inhibition on malate or glutamate dehydrogenases. It should be noted that CSA did not protect from the inhibitory action of the steroid on oxygen consumption (not shown).

Sensitivity to the immunosuppressant CSA is an important characteristic of the permeability transition process [21–24]. The experiment of Fig. 3A illustrates that, certainly, the addition of 0.5 μM CSA inhibits the membrane leakage promoted by 166 μM DHEA. Fig. 3A also shows that succinate reversed, to some extent, Ca^{2+} efflux; however, after a period of a few minutes, the release of Ca^{2+} started again. Nevertheless, a complete uptake of Ca^{2+} was attained after the addition of CSA. Fig. 3B shows that 0.05% bovine serum albumin, added during the Ca^{2+} efflux phase, entirely restored mitochondrial Ca^{2+} uptake.

The experiments described in Fig. 4 were undertaken to analyze the effect of DHEA on the transmembrane electric gradient. Panel A depicts that the addition of 0.166 μM DHEA to mitochondria, treated with 50 μM Ca^{2+}, induced a drop in $\Delta\Psi$. It is also shown that succinate and CSA were unable to restore $\Delta\Psi$ to a high level. Bobyleva et al. [25] have shown that DHEA induces mitochondrial proton leakage, Thus, a question arises about the requirement for Ca^{2+} to achieve the effect of DHEA on membrane permeability. To answer such a question DHEA was added previously to Ca^{2+} addition. As observed in Panel B the steroid per se caused a slight diminution in the level of membrane potential, which attained its lowest level after the addition of 50 μM Ca^{2+}. Thus, Ca^{2+} resulted indispensable for the effect of DHEA on membrane permeability transition. Panel C shows that CSA partially abolished the effect of the steroid on $\Delta\Psi$. In Fig. 4 it is also observed that succinate oxidation was not able to restore membrane potential to its original level. Panel D illustrates that, similarly to what it does to Ca^{2+} efflux, 0.05% BSA completely reversed the effect of DHEA on $\Delta\Psi$.

In the experiment described in Fig. 5, mitochondrial swelling was followed as a variable to estimate DHEA-induced permeability transition. It is shown that after the addition of Ca^{2+}, 166 μM DHEA induced a fast increase in mitochondrial volume; this response was arrested by the addition of CSA. It is also shown that BSA inhibited the swelling but did not reverse it.

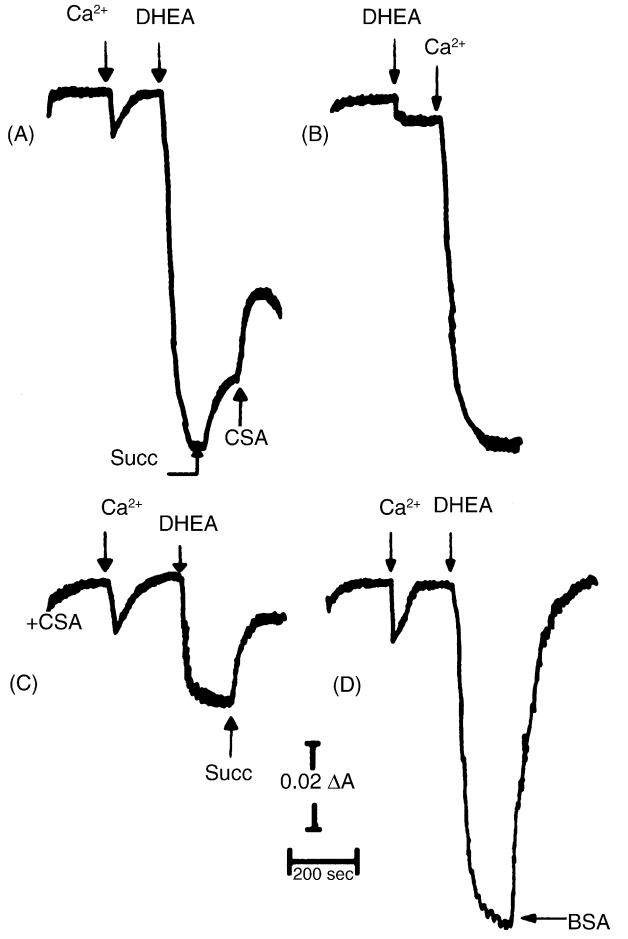

Fig. 4. Effect of succinate, CSA, and BSA on the collapse of $\Delta\Psi$ by DHEA. Experimental conditions as described for Fig. 1, except for the addition of Safranine instead of Arsenazo III. Where indicated the additions were as follows: 50 μM $CaCl_2$, 166 μM DHEA, 5 mM succinate, 0.5 μM CSA, and 0.05% BSA. Final volume, 3 ml and temperature, 22 °C.

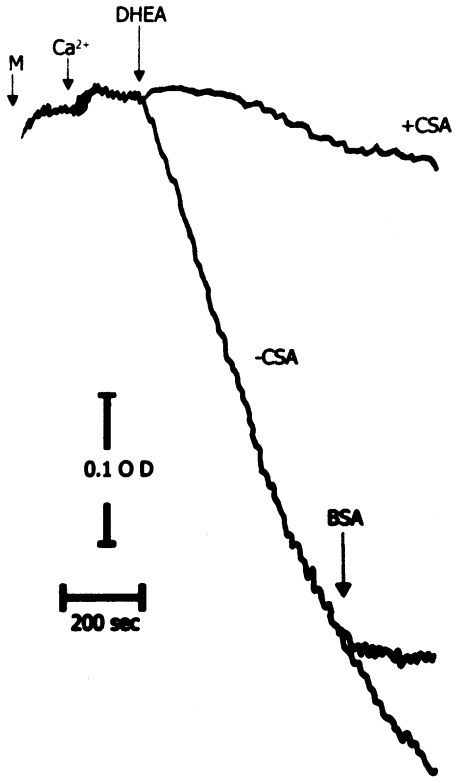

Fig. 5. DHEA-induced mitochondrial swelling. Effect of succinate, CSA, and BSA. Two mg mitochondrial protein were incubated in a basic medium as described under Section 2. The 50 μM $CaCl_2$, 166 μM DHEA, 0.5 μM CSA, 5 mM succinate, and 0.05% BSA were added where indicated. Final volume, 3 ml and temperature, 22 °C.

Inhibition of the electron transport leads to the formation of reactive oxygen species, which eventually induces lipid peroxidation [20,24]. This process conduces to permeability transition [11–13]. The experiment shown in Table 1 was performed in order to ascertain whether DHEA induced membrane injury by promoting an increase in thiobarbituric acid-reactive species (TBARS). Certainly, an increase in DHEA concentrations corresponded to an increase in TBARS, i.e., from 0.049 ± 0.016 nmol/mg in control mitochondria, to 0.157 ± 0.035 nmol/mg in mitochondria treated with 200 μM DHEA.

Table 1
Effect of increasing concentrations of DHEA on TBARS formation

DHEA (μM)	TBARS (nmol/mg)
0	0.049 ± 0.016
100	0.083 ± 0.030
150	0.129 ± 0.015
200	0.157 ± 0.035

Experimental conditions were identical to those of Fig. 1. Values represent averages of four different experiments ± S.D.

At this stage of the experimental work it was decided to explore if treating the rats, in vivo, with a high dosage (120 mg/kg body weight) of the hormone reproduced the DHEA in vitro-induced permeability transition. To this purpose, Ca^{2+} uptake and release were analyzed in kidney mitochondria isolated from DHEA-treated rats. Fig. 6 trace A, illustrates that in mitochondria from treated rats, Ca^{2+} accumulated in a similar way as it did in control mitochondria; however, after a short period of time the cation was released, indicating membrane leakiness. Trace B shows that this response was inhibited by CSA.

4. Discussion

The results of this study show that DHEA, a steroid of the adrenal cortex, induced permeability transition in mitochondria isolated from rat kidney. As a manifestation of permeability transition, matrix Ca^{2+} release and collapse of the transmembrane electric gradient were analyzed. Previous reports on the action of DHEA on mitochondrial functions showed that the steroid inhibits the oxidation of NAD-dependent substrates [7,8]. Here we show that the

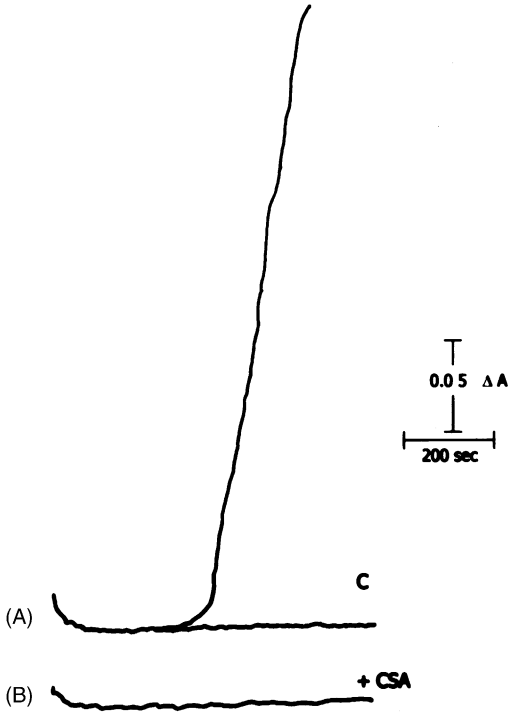

Fig. 6. Calcium movement in mitochondria isolated from DHEA-treated rats. Experimental conditions as described in Fig. 1. Treated rats received injections, i.p. of 120 mg DHEA/kg body weight during 2 days, and then kidney cortex mitochondria were isolated. Where indicated 0.5 μM cyclosporin A (CSA) was added.

target site for the steroid appears to be NADH–CoQ span of the respiratory chain. Since in our experiments the energy requirement is supported by the oxidation of malate and glutamate, it would appear reasonable to assume that Ca^{2+} release, and drop in $\Delta\Psi$ may be due to the inhibition of the electron transport chain. However, two points must be taken into account: (i) the experiments on the collapse of $\Delta\Psi$ pointed out that Ca^{2+} is required to attain the effect of the steroid. As it is known, matrix Ca^{2+} load is a prerequisite for the opening of the large conductance channel, in addition to an inducing agent [26] and (ii) Ca^{2+} efflux, collapse of $\Delta\Psi$, and mitochondrial swelling were sensitive to the immunosuppressant CSA. Accordingly, the effects of DHEA on membrane permeability might be due to the opening of the non-specific pore. It should be mentioned that in comparison with another inducing agents of pore opening, the concentration of DHEA is high. However, it must be stressed that DHEA therapy requires high doses [3].

As illustrated, BSA reversed the effects of the steroid hormone on membrane injury. Regarding this latter, previous works have shown that serum protein inhibits permeability transition by a mechanism involving the redox state of the respiratory chain [27]. Nevertheless, largely because BSA reverses the drop in permeability transition as well as Ca^{2+} release, we propose that DHEA interacts superficially with the lipid milieu of the inner membrane, in such a way that BSA can dissociate it, thus, closing the pore.

An arrest in the rate of electron transport induces permeability transition. In a previous work by our group it was demonstrated that the addition of antimycin A, an inhibitor of the bc1 complex, promotes the aperture of the non-specific pore [23]. In addition, it has been shown that rotenone, an inhibitor at Site I, also induces membrane leakage [19]. It seems that the mechanism involved is related to a stimulated generation of reactive oxygen species. The oxygen-derived free radicals attack the lipid phase of the inner membrane; thus, the membrane oxidative damage increases non-specific permeability. From the results indicating the rotenone-like effect of DHEA, and that the steroid induced the formation of TBARS, we may suggest that the mechanism involved in the induction of membrane permeability is related to the production of reactive oxygen species.

Finally, it should be noted that DHEA promotes diverse deleterious effects in cells, among them apoptosis [5]. Since mitochondrial permeability transition appears to be the primary event in apoptosis [28,29], the results of this work would explain the mechanism by which DHEA is an apoptosis inducer.

Acknowledgements

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