Effect of dehydroepiandrosterone on central and peripheral levels of allopregnanolone and β-endorphin

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Objective: To evaluate the effects of dehydroepiandrosterone (DHEA) oral administration on neuroendocrine function by investigating the modulation exerted by DHEA administration on allopregnanolone and β-endorphin (β-EP) central and peripheral levels in ovariectomized rats.
Design: Prospective study.
Setting(s): Experimental research environment.
Animal(s): Female Wistar rats (n = 48).
Intervention(s): Forty rats were ovariectomized and received an oral treatment with either placebo or 0.5, 1, or 2 mg/kg/day of DHEA. After euthanization, β-EP levels were measured in hippocampus, hypothalamus, anterior pituitary, neurointermediate pituitary, and plasma. Allopregnanolone and DHEAS levels were measured in hippocampus, hypothalamus, anterior pituitary, adrenal glands, and serum. Serum E_2 concentration was also measured.
Main Outcome Measure(s): Dehydroepiandrosterone sulfate ester (DHEAS), E_2, β-EP, and allopregnanolone levels.
Result(s): Dehydroepiandrosterone administration increased DHEAS content in all organs and serum, except for anterior pituitary, where no significant changes occurred. DHEA administration in ovariectomized animals did not significantly increase E_2 circulating levels. DHEA administration induced an increase in allopregnanolone and β-EP content in hippocampus, hypothalamus, and anterior pituitary and in serum or plasma.
Conclusion(s): Dehydroepiandrosterone administration in ovariectomized animals increased allopregnanolone and β-EP central and peripheral levels, which suggests that this compound may play a role as a neuroendocrine mediator, possibly substantiating the beneficial effects of postmenopausal DHEA therapy on the central nervous system. (Fertil Steril® 2005;83(Suppl 1):1161–8. ©2005 by American Society for Reproductive Medicine.)

Key Words: Estradiol, DHEAS, DHEA, allopregnanolone, β-endorphin, rat, ovariectomy, neurosteroids, opioids

Recently, attention has been focused on hormonal replacement therapies alternative to the classic estrogen-progestin one. In particular, dehydroepiandrosterone (DHEA) has been studied in view of its ability to improve sexual and mood disorders, neuroendocrine dysfunction, and metabolic and bone mass changes in postmenopausal women (1–5). DHEA and its sulphate ester (DHEAS) are the major circulating products of adrenals, which is the principal source of these steroids; 20% of circulating DHEA is also produced by ovarian thecal cells under the control of LH and by the central nervous system (CNS), as indicated by the data obtained in ovariectomized and adrenalectomized rats (6–8).

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DHEA levels rise progressively from prepuberty to the 3rd decade of life; then they start to decrease independently from the menopausal transition, reaching 20% of the maximum plasma concentration after 70 years of age (9).

Experimental data suggest that DHEA exerts its effects on the CNS through an antagonist action on γ-aminobutyric $acid_A$ ($GABA_A$) receptor in a dose-dependent manner, with a consequent increase in neuronal excitability (10). In this way, DHEA is able to improve physical and psychological well-being and memory performances in the elderly (11). In fact, in experimental animals, DHEA treatment induces a memory-enhancing effect, and in vitro studies suggest a neurotrophic effect on neurons and glial cells (12). Recent studies have investigated the endocrine and neuroendocrine impact of 6-month 50-mg and 12-month 25-mg daily DHEA administration in early and late postmenopausal women. These results have reported, respectively, increased concentrations of supraphysiological and physiological values in

young women of estrogens, Δ-4 androgens, and Δ-5 androgens, but also of P, allopregnanolone, and β-endorphin (β-EP) (5, 13), indicating a possible role of DHEA as a modulator of neuroendocrine function.

Allopregnanolone is a 3-α, 5-α reduced metabolite of P (14), and its major sources are the gonads and adrenal cortex, and, to a lesser extent, the CNS (15, 16). Allopregnanolone acts as an agonist of $GABA_A$ receptor, modulating stress, mood, and behavior (17, 18). Gonadal steroids may modulate allopregnanolone levels, as suggested by several experimental studies on animal models. In fact, female rats show significantly higher hippocampal allopregnanolone concentrations on the morning and afternoon of proestrous than at diestrous or estrous, reaching the lowest levels at estrous (15). Moreover, ovariectomy determines an increased adrenal allopregnanolone content and a reduction in allopregnanolone levels in brain and serum; this may be due to an estrogen-mediated enzymatic induction in the synthesis of allopregnanolone (19–21).

A direct effect of allopregnanolone on ovulatory function has also been demonstrated in rats by the inhibitory action of intracerebroventricular administration of allopregnanolone on ovulation (22). In addition, recent data seem to confirm that the effects of allopregnanolone on $GABA_A$ receptors are influenced by ovarian hormones (23); estrogen and P may regulate GABA responses, that is, the synthesis and the turnover of $GABA_A$ receptors, through a long-term genomic action (24, 25).

β-endorphin is the most important and biologically active endogenous opioid peptide; it has behavioral, analgesic, thermoregulatory, and neuroendocrine properties. A decrease in central and peripheral β-EP levels in ovariectomized rats and in circulating β-EP levels in postmenopausal women has been shown (26, 27). This postmenopausal reduction in circulating β-EP has been suggested to have a role in the mechanisms of hot flashes and sweats episodes and in the pathogenesis of mood, behavior, and nociceptive disturbances. Indeed, a positive effect of hormone therapy on vasomotor and subjective psychobehavioral symptoms may be mediated by acting on the opiatergic pathway (26). In postmenopausal women, transdermal E_2, independently from the type of progestin associated, induces a significant increase in plasma β-EP levels to premenopausal values and restores the β-EP response to naloxone and clonidine tests, which is impaired in postmenopausal women (20).

In the animal model, estrogens (17β-E_2, conjugated equine, estrone sulphate, and E_2 valerate) (28) and selective estrogen receptors modulators (LY-117018 and EM-652) (21, 29) are able to change central and peripheral levels of allopregnanolone and β-EP, reversing the modifications induced by ovariectomy in female rats. These data have suggested that the effects of estrogenic therapy on the neuroendocrine milieu may also be mediated by the neurosteroid and opioidergic pathways.

The aim of the present study was to evaluate the effects of DHEA oral administration on neuroendocrine function by investigating the modulation exerted by DHEA administration on allopregnanolone and β-EP central and peripheral levels in ovariectomized (ovx) rats.

MATERIALS AND METHODS

Animals

Female Wistar rats (48 healthy cycling 16-week-old rats weighing 155–200 g) were included in the present study. One group of eight cycling rats were included as controls. All rats had 14 hours per day of illumination (light on at 6 A.M. and off at 8 P.M.) and free access to standard rat chow and tap water. Cycling rats were all housed together, and vaginal smears were performed in two cycles; 40 rats were then ovariectomized at the same estrous cycle stage, as assessed by vaginal smears. Ovx rats were treated for 14 days with three different doses of DHEA (according to the following protocol).

Protocol

Ovariectomized animals were housed for 14 days for acclimatization and were then divided into four groups of eight rats each, receiving a 14-day oral treatment with either placebo or 0.5, 1, or 2 mg/kg/day of DHEA.

Twenty-four hours after the last treatment, each animal was euthanized by decapitation (on the same day) under deep pentobarbital anesthesia (30 mg/kg IP), as described elsewhere (26, 30). Cycling control rats were sacrificed on the proestrous morning, as verified by a vaginal smear.

A blood specimen was drawn from each rat in heparinized and nonheparinized tubes, as reported elsewhere (26, 30). Blood collected in heparinized and nonheparinized tubes was centrifuged at 3,500 rpm for 10 minutes, and plasma/serum was stored at −20°C until assay. The following organs were carefully dissected using the optic microscope: anterior pituitary, neurointermediate pituitary, hypothalamus, hippocampus, and adrenal glands. All organs were weighed, collected in a 2.5-mL solution of 4% acetic acid, and homogenized at ice-cold temperature. The homogenate was centrifuged at 1,200 rpm for 15 minutes at 4°C, and the supernatant was divided in two aliquots (1.25 mL each) and assayed in duplicate for allopregnanolone and β-EP. β-EP levels were measured in hippocampus, hypothalamus, anterior pituitary, neurointermediate pituitary, and plasma, while allopregnanolone levels were measured in hippocampus, hypothalamus, anterior pituitary, adrenal glands, and serum within 60 days from sacrifice.

The protocol was approved by the local Institutional Review Board.

β-Endorphin Assay

The supernatant of tissue homogenates and plasma was passed through a C-18 Sep-Pak cartridge, which was previ-

ously equilibrated with 50% aqueous methanol, and the unconjugated fraction was eluted with absolute methanol and brought to dryness under vacuum. β-EP levels were measured by a previously described specific radioimmunoassay (26, 28), using camel β-EP as a standard (Sigma, St. Louis).

The antiserum (supplied by Dr. P. Sacerdote, Milan, Italy) was used at the final dilution of 1:130.000. Analytical grade solvents were purchased from Merck (Darmstadt, Germany); C-18 Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). The sensitivity of this assay is 10 pg/mL; the recovery after acetic acid extraction and chromatography corresponded to 85% ± 11% of the total amount, and the intra- and interassay coefficients of variation were 6% and 8%, respectively. The protein content was determined on the whole homogenate by the Bradford method; the protein content and the weights of the organs of each group of rats were not significantly different. In accordance with previously reported data, β-EP levels were expressed in nanograms/organ in all tissues and in nanograms/milliliter in plasma (28, 31–33).

Allopregnanolone Assay

The supernatant of tissue homogenates and serum was passed through a C-18 Sep-Pak PLUS cartridge, which was previously equilibrated with homogenizing buffer. The cartridge was sequentially washed with homogenizing buffer and 50% aqueous methanol, and the unconjugated steroid fraction was eluted with absolute methanol and brought to dryness under nitrogen. Analytical grade solvents were purchased from Merck; C-18 Sep-Pak PLUS cartridges were obtained from the Waters Corporation. Allopregnanolone contents were measured by a radioimmunoassay (RIA) method described elsewhere, using an antiserum kindly provided by Dr. R. H. Purdy (San Diego, CA) (29, 34). The sensitivity of this assay was 15 pg/mL, the recovery after extraction and chromatography was 86.5 % ± 12.7% (mean ± SEM), and the intra- and interassay coefficients of variation were 7% and 9%, respectively (34). In accordance with previously reported data, allopregnanolone levels were expressed in picograms/milligram of tissue in each tissue and in picograms/milliliter in serum (15, 16, 34).

DHEAS Assay

The supernatant of tissue homogenates and serum was passed though a C18 Sep-Pak cartridge, which was previously equilibrated with homogenizing buffer. The DHEAS contents were measured by specific RIA. The sensitivity of the assay was 0.16 ng/mL, and the intra- and interassay coefficients of variation were 6.2% and 8.5%, respectively.

Estradiol Assay

The serum was passed through a C18 Sep-Pak cartridge, which was previously equilibrated with homogenizing buffer. E_2 contents were assayed by a specific commercially available RIA kit. The sensitivity of the assay was 10 pg/mL, and the intra- and interassay coefficients of variation were 2.5% and 3.5%, respectively.

Statistical Analysis

Data are reported as mean ± SD. Comparison among different treatment groups was performed by means of one-way analysis of variance or the Kruskal-Wallis test, as appropriate. Differences between single pairs of groups were analyzed by means of the paired Student's *t*-test.

RESULTS

DHEAS

Dehydroepiandrosterone administration increased DHEAS content in a dose-dependent manner in hippocampus, hypothalamus, and serum (Fig. 1). In hippocampus and hypothalamus, only the 2.0 mg/kg/day dose induced an increase in DHEAS levels with respect to fertile rats, while at the lowest doses, DHEAS levels did not differ from those of fertile rats (Fig. 1). At the highest DHEA dose, DHEAS levels were higher than in fertile animals in all organs ($P<.05$), except for the anterior pituitary, where no significant changes occurred (Fig. 1). DHEA administration at 0.5 mg/kg/day induced a decrease in adrenal DHEAS levels in comparison with fertile ($P<.01$), ovx ($P<.05$), 1.0 mg/kg/day ($P<.01$), and 2.0 mg/kg/day ($P<.001$) (Fig. 1).

Estradiol

Dehydroepiandrosterone administration in ovx animals at all doses did not significantly increase E_2 circulating levels (12.0 ±1.6 pg/mL in ovx animals; 12.8 ± 2.6 pg/mL in ovx animals treated with DHEA 0.5 mg/kg/day, P = NS vs. ovx; 13.4 ± 2.3 pg/mL in ovx animals treated with DHEA 1 mg/kg/day, P = NS vs. ovx; 14.2 ± 1.8 pg/mL in ovx animals treated with DHEA 2 mg/kg/day, P = NS vs. ovx).

β-Endorphin

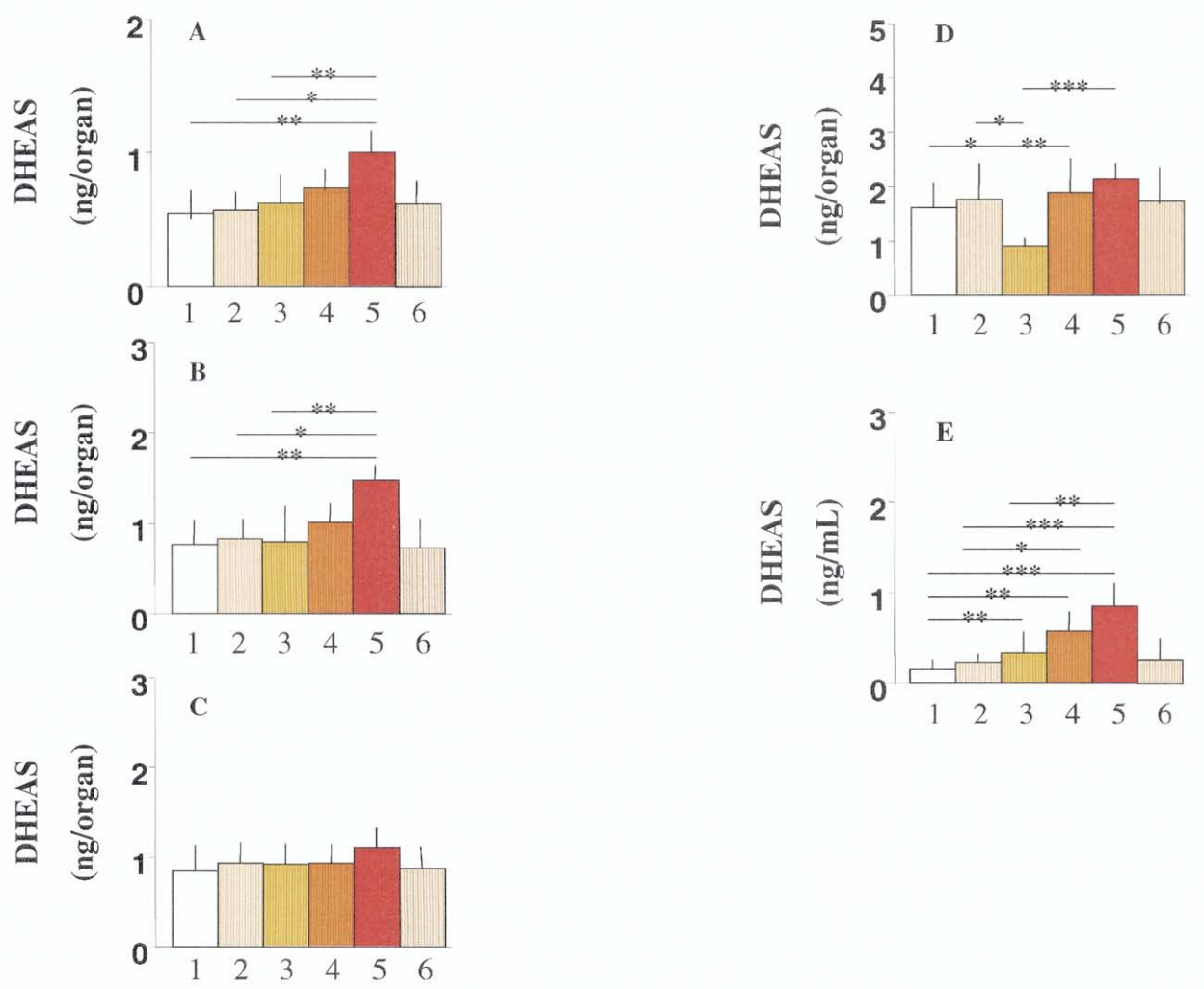
Dehydroepiandrosterone administration induced an increase in β-EP content in hippocampus, hypothalamus, anterior and neurointermediate pituitary ($P<.01$), and plasma in a dose-dependent manner (Fig. 2). At the highest dose, DHEA administration induced an increase in β-EP plasma levels ($P<.001$ vs. ovx), to those observed in fertile animals, and an even greater increase in the hypothalamus ($P<.001$ vs. ovx and fertile) (Fig. 2).

Allopregnanolone

Dehydroepiandrosterone administration induced an increase in hypothalamus ($P<.01$), anterior pituitary ($P<.001$), and serum ($P<.001$) allopregnanolone content in a dose-dependent manner (Fig. 3). DHEA administration at the highest dose increased hippocampus allopregnanolone content ($P<.001$),

FIGURE 1

Dehydroepiandrosterone sulfate ester (DHEAS) central and peripheral levels in fertile and ovx rats before and after DHEA (three doses) and placebo treatment. (**A**) Hippocampus; (**B**) hypothalamus; (**C**) anterior pituitary; (**D**) adrenal; (**E**) serum. *Column 1*, fertile rats; *column 2*, ovx rats; *column 3*, ovx rats treated with DHEA (0.5 mg/kg/day); *column 4*, ovx rats treated with DHEA (1.0 mg/kg/day); *column 5*, ovx rats treated with DHEA (2.0 mg/kg/day); *column 6*, ovx rats treated with placebo. *$P<.05$; **$P<.01$; ***$P<.001$.



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restoring allopregnanolone levels to levels similar to those observed in fertile rats (Fig. 3). Adrenal allopregnanolone content did not change in response to DHEA therapy (Fig. 3).

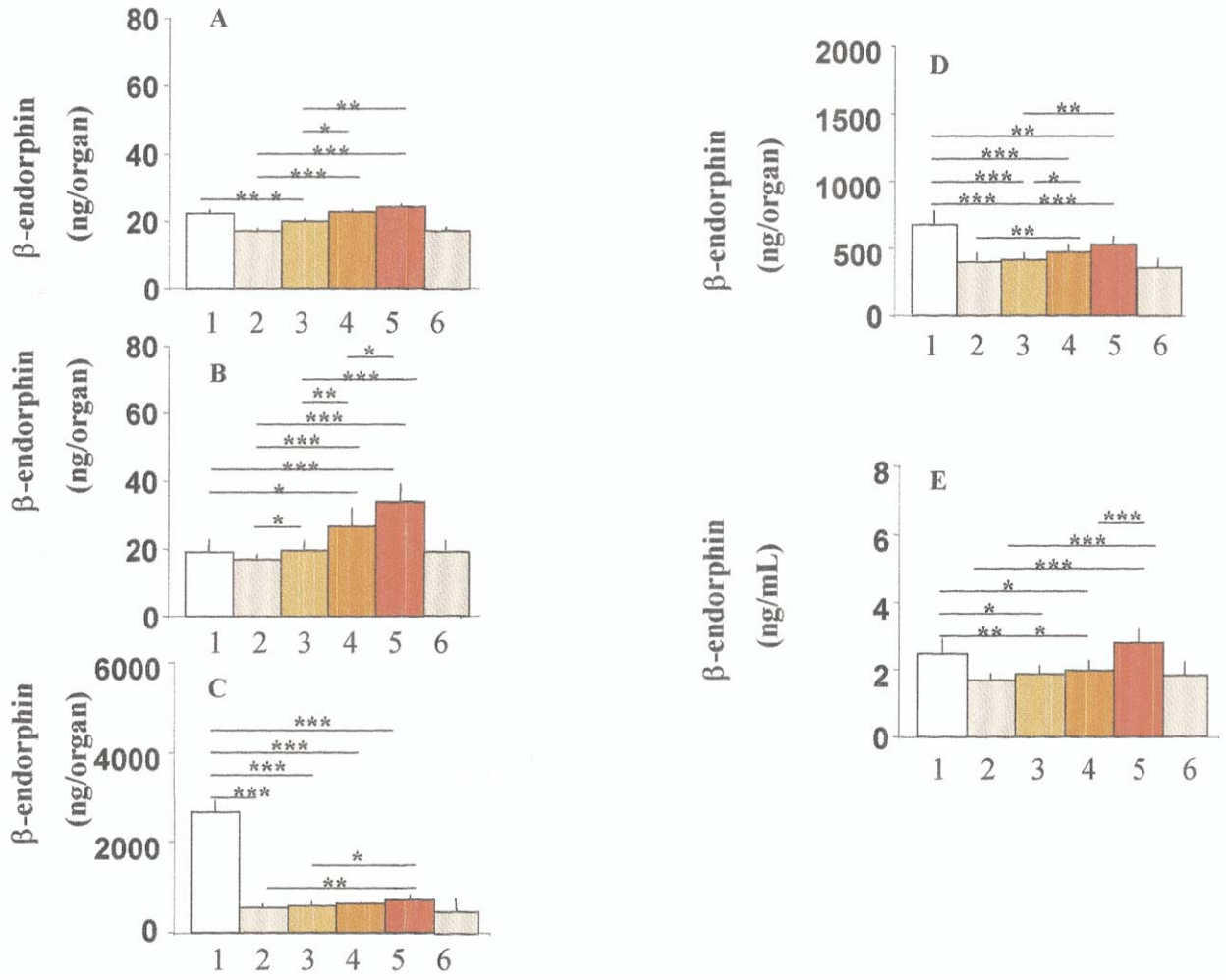
DISCUSSION

In the last 20 years, the increase in human life expectancy has stimulated numerous research groups to investigate the pathophysiology of brain senescence and to find pharmacological compounds able to counteract these processes (35). Several studies have been performed to evaluate whether estrogens are involved in the aging process (36, 37). On the other hand, there is an increasing awareness of the significant activity of endogenous or exogenous androgens because in women middle age is characterized by the coexistence of menopause and adrenopause.

Among androgen molecules, only a few studies have been conducted to investigate the physiological function of DHEA(S) and the possible role of this molecule on the CNS in humans and in animals. DHEA administration at 50 mg/day induced an improvement in psychological and physical well-being in postmenopausal women (38, 39), thus suggesting a specific role for DHEA supplementation on CNS functions. Recently, it has been shown that the oral administration of DHEA (50 mg/day) tends to determine an in-

FIGURE 2

β-endorphin (β-EP) central and peripheral levels in fertile and ovx rats before and after dehydroepiandrosterone (DHEA) (three doses) and placebo treatment. (**A**) Hippocampus; (**B**) hypothalamus; (**C**) neurointermediate lobe; (**D**) anterior pituitary; (**E**) plasma. For explanation, see Figure 1. *$P<.05$; **$P<.01$; ***$P<.001$.



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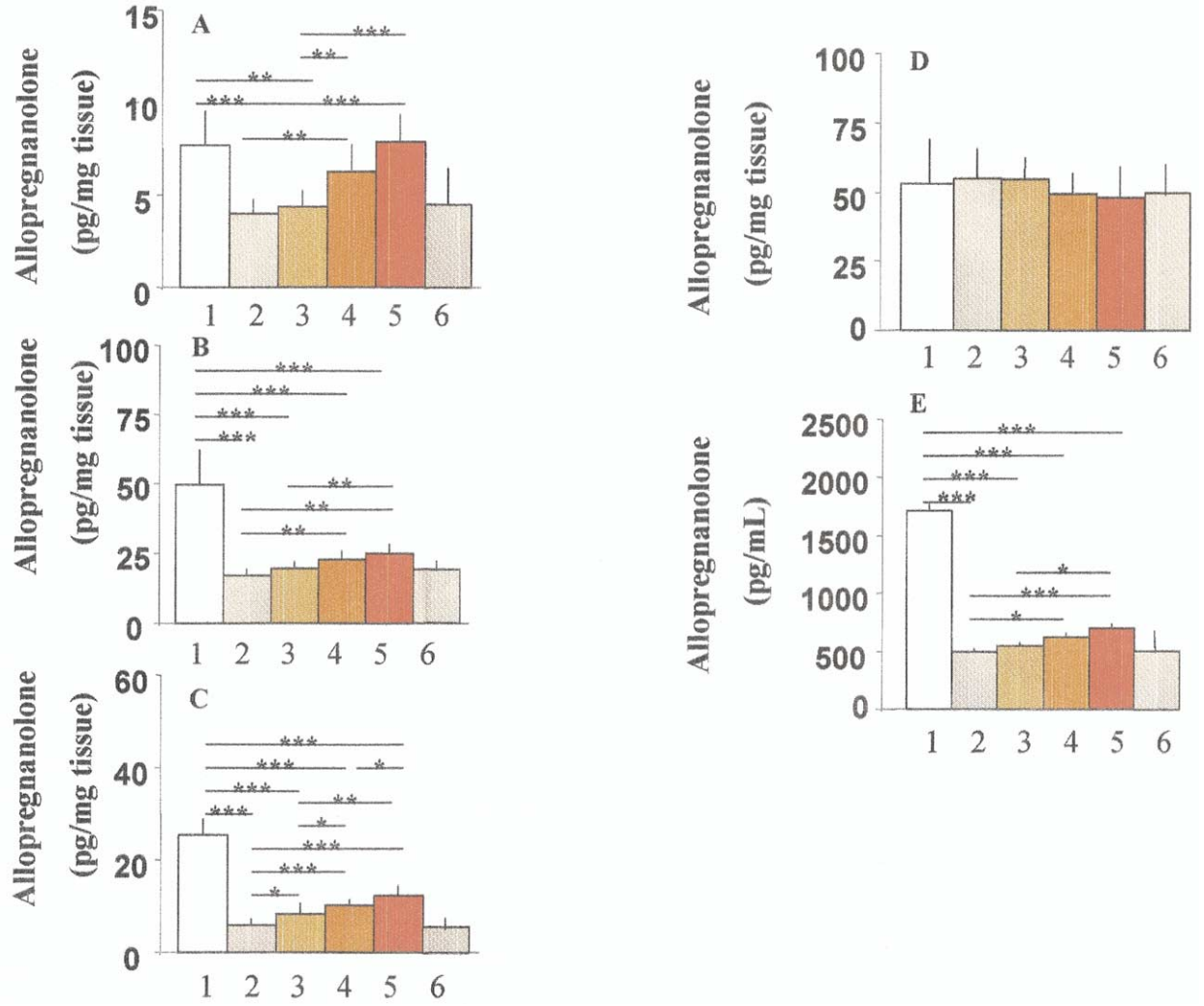
crease in well-being and mood only in women (40). Moreover, another study described DHEA(S) as having an antidepressant function (30–90 mg/day for 4 weeks) in middle-aged and elderly patients with major depression and low basal DHEA(S) levels (3). DHEA administration, like estrogen, has been reported to revert postmenopausal changes on opiatergic, adrenergic, and serotoninergic pathways, restoring the β-EP response to clonidine, naloxone, and fluoxetine tests and increasing circulating β-EP levels (4, 20, 41–43).

The present study evaluated the effects of DHEA administration on central and peripheral β-EP and allopregnanolone content in ovx rats. According to previous experimental evidence, estrogen deprivation induces a decrease in central and peripheral β-EP levels in ovx rats (26, 28, 31, 33). The ovariectomy-induced β-EP decrease varies in the different examined tissues, in agreement with previous data (28, 31, 33). This is probably related to a different sensitivity of the examined areas to estrogens or to a different expression of estrogen receptor subtypes (α and β) (44–46).

Dehydroepiandrosterone administration induces an increase in β-EP content in anterior and neurointermediate pituitary and hippocampus in a dose-dependent manner. High-dose DHEA administration induces an increase in β-EP circulating levels to those observed in fertile animals and an even greater increase in the hypothalamus. Comparing these results with those assessed administering E_2 in ovx rats, we can observe several similarities. In all the examined organs, the effect of DHEA on β-EP levels closely reflects that of E_2 valerate, with the exception of the neurointerme-

FIGURE 3

Allopregnanolone central and peripheral levels in fertile and ovx rats before and after dehydroepiandrosterone (DHEA) (three doses) and placebo treatment. (**A**) Hippocampus; (**B**) hypothalamus; (**C**) anterior pituitary; (**D**) adrenal; (**E**) serum. For explanation, see Figure 1. *$P<.05$; **$P<.01$; ***$P<.001$.



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diate pituitary, where DHEA induces a lower increase in β-EP.

Dehydroepiandrosterone administration induces an increase in allopregnanolone content in the hypothalamus, anterior pituitary, serum, and hippocampus, where DHEA administration restores allopregnanolone levels to those observed in fertile rats. Similar data have been observed at the central level when administering E_2 valerate in ovx rats. However, DHEA does not determine a decrease in allopregnanolone adrenal content, as observed with E_2. Moreover, the serum allopregnanolone levels reached in response to DHEA therapy are lower than those obtained with E_2 administration.

The mechanisms of action of DHEA are not clear. Part of the effects of DHEA depends on its conversion to estrogens and androgens and on the recruitment of their receptors. In fact, at the vascular level, a reduction of atherosclerotic lesions by DHEA in rabbits was reported, partially through conversion to estrogens (47). However, there is evidence that DHEA may have a specific receptor. This has been demonstrated in blood vessels, where DHEA binds with high affinity to nonhuman endothelial cell membranes without being displaced by structurally related steroids (48).

In the brain, DHEA and DHEAS are produced by glial cells, and experimental evidence suggests that their CNS effects may be mediated by a specific binding to the $GABA_A$ receptor, thus blocking the GABA-induced chloride trans-

port or current in synaptoneurosomes and neurons, in a dose-dependent manner, with an increase in the neuronal excitability (10). Moreover, a potentiating effect of DHEA on N-methyl-D-aspartate and sigma receptors has been reported in the rat brain (49).

The present data seem to indicate that the effect of DHEA administration may not be entirely ascribed to the conversion in estrogenic metabolites. In fact, the E_2 levels reached in DHEA-treated ovx animals were not significantly different from those observed in untreated ovx animals and significantly lower than those obtained in ovx animals in response to E_2 valerate administration. Similar levels of β-EP and allopregnanolone, obtained in various organs, were observed in response to the two different therapies. Therefore, DHEA may act directly or through its local metabolites on neurosteroidogenesis and the opioidergic pathway.

In conclusion, the changes of central and peripheral allopregnanolone and β-EP levels in response to DHEA in ovx animal models suggest that this compound may play an important role as a neuroendocrine mediator. This evidence represents a biologic basis to substantiate the beneficial effects of DHEA on climacteric symptoms, memory, cognition, and well-being in postmenopausal women.

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