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Conjugated equine estrogens reverse the effects of aging on central and peripheral allopregnanolone and beta-endorphin levels in female rats

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Objective: To compare β-endorphin and allopregnanolone levels and their response to a 2-week oral estrogen treatment with conjugated equine estrogens (CEE) in young ovariectomized (ovx) and in healthy aged female rats.

Design: Prospective study.

Setting: Animal laboratory in an academic environment.

Animal(s): Twenty-four young ovx and 24 healthy aged female Wistar rats were treated with CEE. Three 8-rat control groups (cycling, ovx, and aged rats) were also included.

Intervention(s): Treated rats underwent 14-day oral treatment with three doses of CEE: 0.1 mg/kg/day, 0.5 mg/kg/day, and 2 mg/kg/day.

Main Outcome Measure(s): Cerebral and peripheral β-endorphin and allopregnanolone levels.

Result(s): Beta-endorphin levels were lower in aged vs. cycling and ovx control rats. In brain and serum allopregnanolone levels were lower in aged vs. cycling control rats, whereas in the adrenals they were higher in aged vs. cycling animals. In the hypothalamus and anterior pituitary allopregnanolone levels were lower in ovx vs. aged animals. In both ovx and aged animals, CEE treatment reverted the effects of ovariectomy and aging, in a dose-dependent manner.

Conclusion(s): Aging is associated with a decrease in cerebral and peripheral β-endorphin and allopregnanolone. In hypoestrogenic rats, CEE treatment restores allopregnanolone and β-endorphin content; this indicates a role for these compounds as neuroendocrine mediators of the effects of estrogens. (Fertil Steril® 2004;81(Suppl 1):757–66. ©2004 by American Society for Reproductive Medicine.)

Key Words: Female aged rats, ovariectomized rats, conjugated estrogens, beta-endorphin, allopregnanolone, brain, serum, adrenal

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Recent experimental and clinical evidence shows a relationship between sex steroid hormones and aging processes. In particular, the decrease in estrogen levels at menopause is involved in the pathophysiology of age-associated diseases, such as cardiovascular disease, osteoporosis, cognitive dysfunction, and central neurodegenerative diseases (Alzheimer and Parkinson diseases) (1, 2). The age-associated decline in women's estrogen levels might cause or contribute to cognitive dysfunction, because estrogen affects neuronal survival in brain regions, such as hippocampus and amygdala, which are of crucial importance for learning and memory (1–4). Furthermore, estrogen regulates synapse formation in the hippocampus and induces the activity of choline acetyltransferase, thus affecting acetylcholine production (4).

Clinical studies suggest that estrogen replacement therapy exerts beneficial effects on cognition in postmenopausal women, particularly on verbal memory and attention (5, 6), although these findings have been questioned by recent randomized trials. In fact, in a recent meta-analysis, Le Blanc et al. (7) showed an improvement in cognitive functions only in symptomatic postmenopausal women. Regard-

ing the effects of estrogen replacement treatment on Alzheimer disease prevention, conclusive data are not yet available, because the studies performed have important methodologic limitations (7–9). However, the recent publication of the Cache County study, showing a decreased incidence of Alzheimer disease in postmenopausal women receiving long-term hormone replacement therapy, strongly supports the role of sex steroid hormones in the maintenance of central nervous system function (10). The neuroendocrine mechanisms underlying the beneficial effects of estrogens are still unclear.

Over the last few years, we have been investigating the effects of sex steroid hormones on opioid peptides and neurosteroids. Both opioid peptides and neurosteroids play a relevant role in the modulation of mood and cognitive performances (11–14). The opioid peptides are important markers of neuroendocrine and behavioral functions, and β-endorphin, the most investigated opioid peptide, is modulated by gonadal steroids. Experimental and clinical studies have demonstrated a decrease in central and peripheral β-endorphin levels in castrated animals and in women with surgical or spontaneous postmenopause (15–23). Intraperitoneal E_2 benzoate or 17β-E_2 administration in castrated rats restores β-endorphin in the hippocampus, hypothalamus, neurointermediate and anterior pituitary lobe, and serum, inducing a regular circadian β-endorphin pattern in the medial basal hypothalamus (15–17, 24). In a recent study, it has been shown that oral estrogen supplementation with E_2 valerate, estrone sulphate, and conjugated equine estrogens (CEE) reverts the ovariectomy-induced neuroendocrine changes in hippocampal, hypothalamic, pituitary, and plasma β-endorphin levels in a dose-dependent manner (25).

Allopregnanolone is a neurosteroid produced by the adrenals, ovaries, and central nervous system, which are also the main target organs for sex steroids. This neurosteroid is involved in the regulation of anxiety and mood, acting as a γ-aminobutyric acid-A receptor agonist. Several studies have suggested that sex steroids modulate allopregnanolone synthesis and release (26–28). The intraperitoneal administration of 17-β E_2 in ovariectomized (ovx) rats prevents the ovariectomy-related decrease in allopregnanolone in the hippocampus, hypothalamus, pituitary, and serum (26). We recently demonstrated that oral estrogens are able to regulate allopregnanolone content in all tissues, except for the adrenals, in a dose-dependent manner. Most data concerning the effects of estrogens on β-endorphin and allopregnanolone were obtained with young, castrated animals and might not be predictive of the effects of hormonal replacement therapy in aging women. Furthermore, no data are available on the effects of estrogens in intact aged rats. The aim of the present study was to compare central and peripheral β-endorphin and allopregnanolone levels and their response to a 2-week oral estrogen treatment with CEE in young ovx and in healthy aged female rats.

MATERIALS AND METHODS

Animals

Female Wistar rats (24 healthy 16-month-old [aged] rats, weighing 500–550 g, and 24 ovx 16-week-old [young] rats, weighing 155–200 g) were included in the present study. One group of 8 cycling rats (16 weeks old, weighing 150–200 g), one group of 8 ovx rats (16 weeks old, weighing 150–200 g), and one group of 8 aged female rats (16 months old, weighing 500–550 g) were included as controls. All rats had 14 hours per day of illumination (lights on at 6 AM and off at 8 PM) and free access to standard rat chow and tap water.

Cycling rats were all housed together. Vaginal smears were carried out for all rats in two different cycles. Twenty-four rats were ovariectomized at the same estrous cycle stage, as assessed by vaginal smears. All aged rats were healthy and in anestrus period. Aged and ovx rats were treated for 14 days with different estrogen regimens, according to the following protocol.

Protocol

Aged and ovx animals were housed for 14 days for acclimatization and were then divided into three groups of eight rats each, receiving a 14-day oral treatment with either 0.1 mg/kg/day, 0.5 mg/kg/day, or 2 mg/kg/day of CEE (Wyeth Ayerst, Philadelphia, PA). In each animal, the drug was administered daily by gavage, and the dose of CEE was dissolved in distilled water (1 mL). In ovx rats the treatment was started 14 days after ovariectomy. The three control groups were housed similarly and sham treated. Twenty-four hours after the last treatment, each animal was killed by decapitation (in the same day) under deep pentobarbital anesthesia (30 mg/kg intraperitoneal), as previously described (13–15). Cycling control rats were killed on the proestrous morning, as verified by a vaginal smear.

A blood specimen was drawn from each rat in heparinized and nonheparinized tubes, as previously reported (13–15). 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 under 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 an 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-endorphin. Beta-endorphin levels were measured in hippocampus, hypothalamus, anterior pituitary, neurointermediate pituitary, and plasma, whereas allopregnanolone levels were measured in hippocampus, hypothalamus, anterior pituitary, adrenal glands, and serum, within 60 days from killing.

TABLE 1

Percentage change of β-endorphin and allopregnanolone in aged vs. cycling, aged vs. ovariectomized, and ovx vs. cycling rats in hippocampus, hypothalamus, anterior pituitary, neurointermediate lobe (β-endorphin), adrenal gland (allopregnanolone), and serum/plasma.

	Aged vs. cycling rats	Aged vs. ovx rats	Ovx vs. cycling rats
β-endorphin			
Hippocampus	−40 ± 4*	−19 ± 2*	−27 ± 2*
Hypothalamus	−49 ± 6*	−34 ±*	−23 ± 3*
Anterior pituitary	−74 ± 6*	−51 ± 4*	−47 ± 4*
Neurointermediate lobe	−83 ± 7*	−65 ± 6*	−52 ± 5*
Plasma	−77 ± 8*	−57 ± 6*	−39 ± 5*
Allopregnanolone			
Hippocampus	−46 ± 5*	−13 ± 1	−53 ± 5*
Hypothalamus	−55 ± 8*	−39 ± 2*	−73 ± 8*
Anterior pituitary	−51 ± 7*	−59 ± 3*	−80 ± 7*
Adrenal galnd	+ 30 ± 4*	+ 9 ± 1	+ 23 ± 6*
Serum	−64 ± 8*	−33 ± 6	−73 ± 9*

Note: Values (% change) are presented as mean ± SD; ovx = ovariectomized.
* $P<.05$.

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The protocol was approved by the local institutional review board.

Beta-Endorphin Assay

The supernatant of tissue homogenates and plasma were passed through a cartridge (C-18 Sep-Pak; Waters Corporation, Milford, MA) previously equilibrated with 50% aqueous methanol, and the unconjugated fraction was eluted with absolute methanol and brought to dryness under vacuum. Beta-endorphin levels were measured by a previously described specific RIA (13, 25) using camel β-endorphin as standard (Sigma Chemicals, St. Louis, MO).

Antiserum was used at the final dilution of 1:130,000. Analytic-grade solvents were purchased (Merck, Darmstadt, Germany). The sensitivity of this assay is 10 pg/mL, the recovery after acetic acid extraction and chromatography corresponded to 85% ± 11% (mean ± SEM) 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 (29); 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-endorphin levels were expressed in ng/organ in all tissues and in ng/mL in plasma (18, 19, 24, 25).

Allopregnanolone Assay

The supernatant of tissue homogenates and serum was passed through a cartridge (C-18 Sep-Pak) previously equilibrated with homogenizing buffer. The cartridge was sequentially washed with homogenizing buffer, 50% aqueous methanol, and the unconjugated steroid fraction was eluted with absolute methanol and brought to dryness under nitrogen. Analytic-grade solvents were purchased (Merck). Allopregnanolone contents were measured by a previously described RIA method (25, 30). 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 (30). In accordance with previously reported data, allopregnanolone levels were expressed in pg/mg of tissue in each tissue and in pg/mL in serum (27, 28, 30).

Statistical Analysis

Data are reported as mean plus or minus standard deviation. Comparison among different treatment groups was performed by one-way analysis of variance or of Kruskal-Wallis test, as appropriate. Differences between single pairs of groups were analyzed by the paired *t*-test.

RESULTS

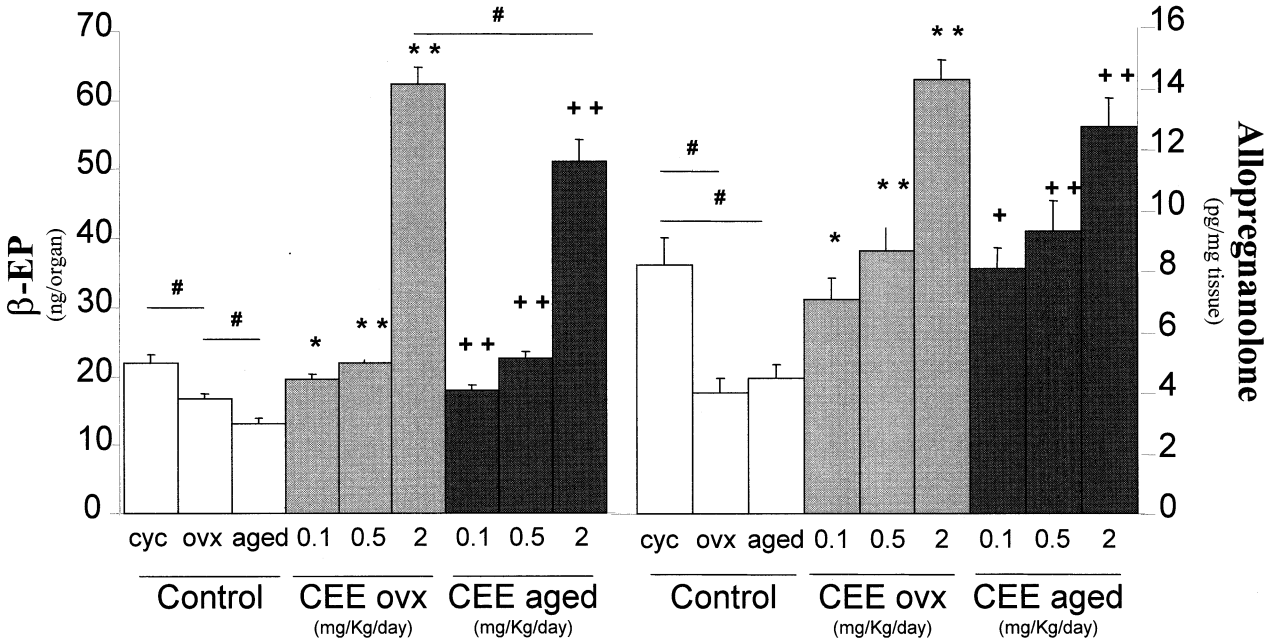
Effects of Aging

In aged animals, β-endorphin levels were significantly lower in all tissues and in plasma compared with those in cycling animals ($P<.01$); the age-related decrease ranged from 40% ± 4% (mean ± SD) in the hippocampus to 83% ± 7% in the neurointermediate lobe (Table 1). The differences between aged and ovx animals were less prominent, ranging from 19% ± 2% in the hippocampus to 65% ± 6% in the neurointermediate lobe (Table 1).

In the hippocampus, hypothalamus, anterior pituitary, and serum allopregnanolone levels were significantly lower in aged than in cycling control rats ($P<.01$), with an age-related decrease ranging from 46% ± 5% in the hippocampus to 64% ± 7% in serum (Table 1). In the adrenal gland, allopregnanolone content was significantly higher (30% ± 4%) in aged vs. cycling animals (Table 1). No significant

FIGURE 1

Beta-endorphin (β-EP) and allopregnanolone levels in the hippocampus of cycling, ovx, and aged female rats. Each group consisted of eight rats. *Left:* Beta-endorphin levels in placebo-treated (control) cycling, ovx, or aged rats (*white bars*), in ovx rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*light grey bars*), and in aged rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*dark grey bars*). *Right:* Allopregnanolone levels in placebo-treated (control) cycling, ovx, or aged rats (*white bars*), in ovx rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*light grey bars*), and in aged rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*dark grey bars*). *$P<.05$ vs. ovx controls; **$P<.005$ vs. ovx controls; $^{+}P<.05$ vs. aged controls; $^{++}P<.005$ vs. ovx controls.



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differences were observed between aged and ovx rats in the hippocampus, adrenal glands, and serum (Table 1), whereas in the hypothalamus (−39% ± 2%) and anterior pituitary (−59% ± 3%) allopregnanolone levels were significantly lower in ovx than in aged animals ($P<.01$) (Table 1).

Effects of CEE Replacement Therapy: Hippocampus

In both aged and ovx rats, CEE treatment determined a significant dose-dependent increase in β-endorphin content, compared with control groups (Fig. 1). Both groups of treated animals showed β-endorphin levels similar to those of cycling rats with the 0.5 mg/kg/day dose, whereas with 2 mg/kg/day they reached significantly higher β-endorphin levels than cycling animals (mean increase of 130% ± 16% and 210% ± 32% in aged and ovx rats, respectively). With CEE at 0.1 and 0.5 mg/kg/day, ovx and aged animals reached similar β-endorphin levels, whereas at 2 mg/kg/day ovx rats showed significantly greater β-endorphin content ($P<.05$) (Fig. 1).

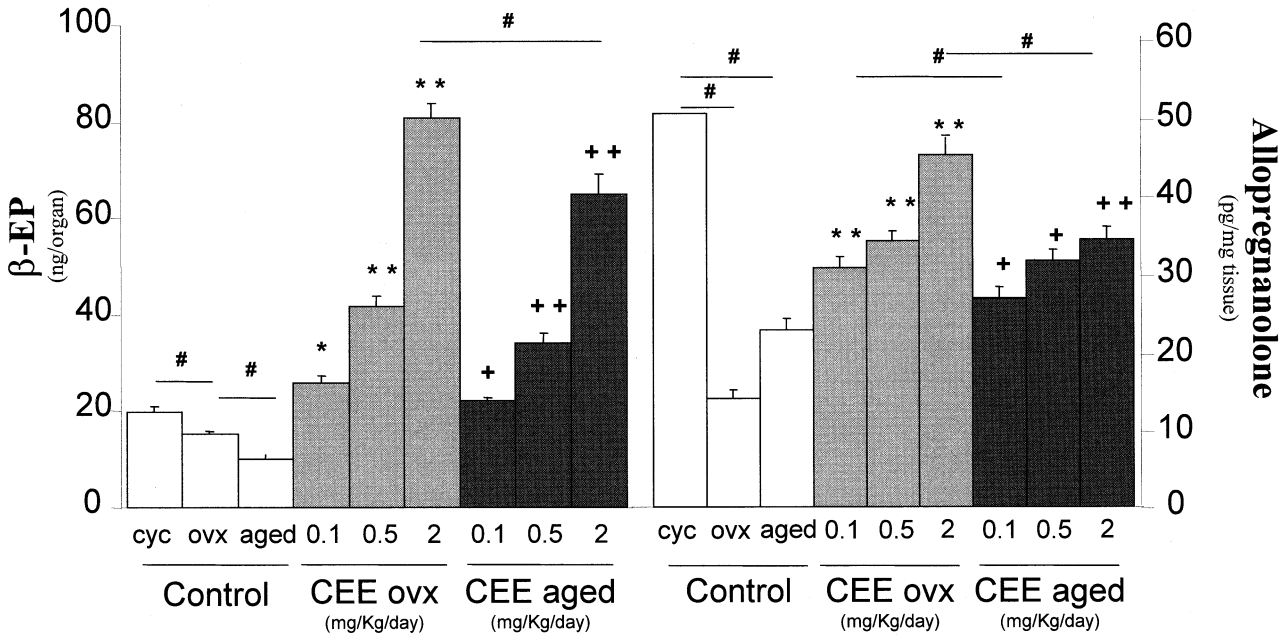
Hippocampal allopregnanolone significantly increased, in a dose-dependent manner, with all doses of CEE in both aged and ovx rats compared with control groups (Fig. 1). At 0.5 mg/kg/day, both groups showed allopregnanolone levels similar to those of cycling animals, whereas CEE treatment at 2 mg/kg/day induced significantly higher β-endorphin levels than in cycling rats (mean increase of 52% ± 10% and 74% ± 16% in aged and ovx rats, respectively) (Fig. 1). No significant differences were observed between aged and ovx treated rats at all CEE dosing regimens (Fig. 1).

Effects of CEE Replacement Therapy: Hypothalamus

Administration of CEE to aged and ovx animals significantly increased β-endorphin content, in a dose-dependent manner, compared with control groups (Fig. 2). Both groups treated at 0.1 mg/kg/day showed β-endorphin levels similar to those of cycling controls, whereas at higher doses they showed significantly higher β-endorphin levels (mean increase of 79% ± 7% at 0.5 mg/kg/day and 242% ± 32% at 2 mg/kg/day in aged rats, and 121% ± 14% at 0.5 mg/kg/day and 305% ± 37% at 2 mg/kg/day in ovx rats). No significant differences were observed between ovx and aged animals at 0.1 and 0.5 mg/kg/day, whereas at 2 mg/kg/day ovx treated animals showed significantly higher β-endorphin levels ($P<.05$) (Fig. 2).

FIGURE 2

Beta-endorphin (β-EP) and allopregnanolone levels in the hypothalamus of cycling, ovx, and aged rats. Each group consisted of eight rats. *Left:* Beta-endorphin levels in placebo-treated (control) cycling, ovx, or aged rats (*white bars*), in ovx rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*light grey bars*), and in aged rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*dark grey bars*). *Right:* Allopregnanolone levels in placebo-treated (control) cycling, ovx, or aged rats (*white bars*), in ovx rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*light grey bars*), and in aged rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*dark grey bars*). *$P<.05$ vs. ovx controls; **$P<.005$ vs. ovx controls; $^{+}P<.05$ vs. aged controls; $^{++}P<.005$ vs. ovx controls.



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Hypothalamic allopregnanolone content significantly increased, in a dose-dependent manner, with all CEE regimens in both aged and ovx rats compared with the corresponding control groups (Fig. 2). Only ovx rats treated with 2 mg/kg/day showed allopregnanolone levels similar to those of cycling animals (Fig. 2). At 0.1 and 2 mg/kg/day, ovx animals showed significantly higher allopregnanolone levels than the corresponding aged rats ($P<.05$) (Fig. 2).

Effects of CEE Replacement Therapy: Anterior Pituitary

Treatment with CEE determined a significant dose-dependent increase in β-endorphin content in aged and ovx rats compared with control groups, reaching levels similar to those of cycling animals at 2 mg/kg/day (Fig. 3). At 0.1 and 0.5 mg/kg/day, ovx animals showed significantly higher β-endorphin levels than the corresponding aged rats ($P<.05$).

Allopregnanolone levels significantly increased, in a dose-dependent manner, with all CEE regimens in aged and ovx rats compared with control groups (Fig. 3). At 2 mg/kg/day, both ovx and aged animals reached levels similar to those of cycling animals (Fig. 3). The aged rats seemed to be more sensitive to a lower dose of CEE because these animals demonstrated a significant increase in allopregnanolone at 0.1 mg/kg/day, whereas the ovx animals demonstrated less responsiveness (Fig. 3).

Effects of CEE Replacement Therapy: Neurointermediate Pituitary

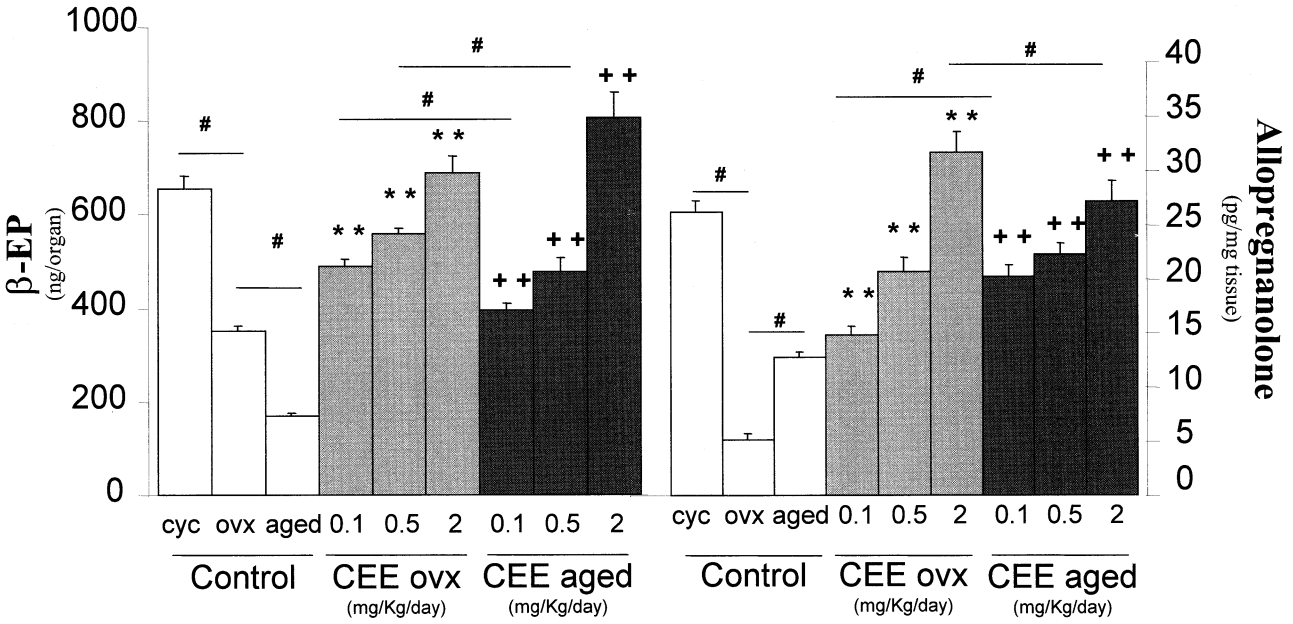
Treatment with CEE determined a significant dose-dependent increase in β-endorphin content in both aged and ovx rats compared with controls (Fig. 4). Ovariectomized animals treated with the lower dose (0.1 mg/kg/day) reached levels similar to those of cycling rats, whereas at 0.5 and 2 mg/kg/day they showed an increase of 40% $\pm$ 3% and 123% $\pm$ 20%, respectively, vs. cycling controls). In aged animals, only the higher dose restored β-endorphin levels to values similar to those of cycling rats (Fig. 4). At each CEE dose, ovx treated animals showed significantly higher β-endorphin levels than the corresponding aged group (Fig. 4).

Effects of CEE Replacement Therapy: Adrenals

Administration of CEE produced a dose-dependent decrease in allopregnanolone content in both aged and ovx rats, reaching statistical significance at 0.5 and 2 mg/kg/day (Fig.

FIGURE 3

Beta-endorphin (β-EP) and allopregnanolone levels in the anterior pituitary of cycling, ovx, and aged female rats. Each group consisted of eight rats. *Left:* Beta-endorphin levels in placebo-treated (control) cycling, ovx, or aged rats (*white bars*), in ovx rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*light grey bars*), and in aged rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*dark grey bars*). *Right:* Allopregnanolone levels in placebo-treated (control) cycling, ovx, or aged rats (*white bars*), in ovx rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*light grey bars*), and in aged rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*dark grey bars*). *$P<.05$ vs. ovx controls; **$P<.005$ vs. ovx controls; $^{+}P<.05$ vs. aged controls; $^{++}P<.005$ vs. ovx controls.



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4). Ovariectomized and aged rats treated with 2 mg/kg/day showed allopregnanolone levels similar to those of cycling animals (Fig. 4). No significant difference was observed between ovx and aged rats treated with 0.1 mg/kg/day, whereas CEE treatment at 0.5 and 2 mg/kg/day induced lower allopregnanolone levels in ovx rats compared with the corresponding aged group (Fig. 4).

Effects of CEE Replacement Therapy: Plasma and Serum

Administration of CEE significantly increased β-endorphin levels, in a dose-dependent manner, in aged and ovx rats compared with control groups (ovx rats: $P<.05$ at 0.1 mg/kg/day and $P<.005$ at 0.5 and 2 mg/kg/day; aged rats: $P<.005$ at all doses). At 2 mg/kg/day, both ovx and aged animals reached allopregnanolone levels higher than those of cycling rats (Fig. 5).

Serum allopregnanolone significantly increased, in a dose-dependent manner, with all CEE regimens in aged and ovx rats compared with control groups (Fig. 5). However, CEE treatment was not able to restore allopregnanolone levels to values present in cycling rats (Fig. 5). Administration of CEE at 0.1 and 0.5 mg/kg/day induced significantly higher allopregnanolone levels in aged rats vs. the corresponding ovx animals (Fig. 5).

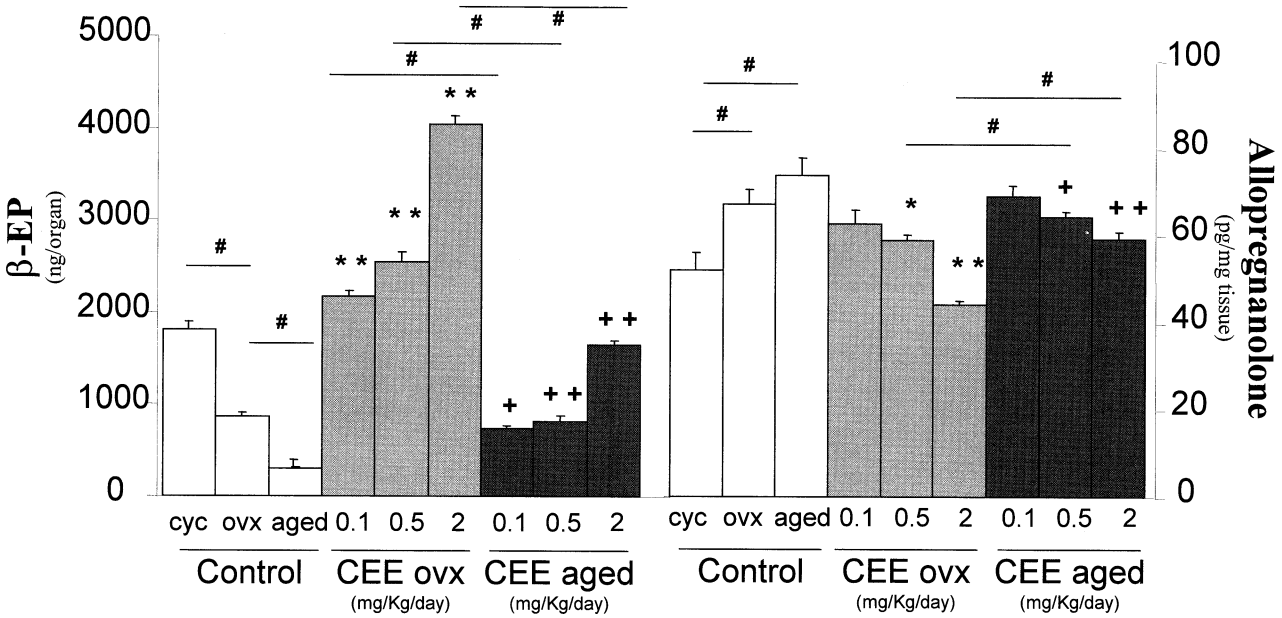
DISCUSSION

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 pharmacologic compounds able to counteract these processes. Several studies have been performed to evaluate whether estrogens are involved in the aging process and whether they can modulate it (4–9, 31–33). Elderly women are more affected by chronic degenerative diseases than age-matched men, and this seems to be related to the long time period of sex steroid hormone deprivation (10).

The present study evaluated the effects of aging on central and peripheral β-endorphin and allopregnanolone content in female rats. In aged animals, β-endorphin levels were significantly lower compared with those in cycling animals in hippocampus, hypothalamus, anterior and neurointermediate pituitary lobe, and in plasma. Moreover, the age-related decrease was more evident than that related to ovariectomy in younger animals. According to previous experimental

FIGURE 4

Left: Beta-endorphin (β-EP) levels in the neurointermediate lobe of cycling, ovx, and aged female rats. Each group consisted of eight rats. Beta-endorphin levels in placebo-treated (control) cycling, ovx, or aged rats (*white bars*), in ovx rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*light grey bars*), and in aged rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*dark grey bars*). *Right:* Allopregnanolone levels in the adrenal gland of cycling, ovx, and aged female rats. Each group consisted of eight rats. Allopregnanolone levels in placebo-treated (control) cycling, ovx, or aged rats (*white bars*), in ovx rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*light grey bars*), and in aged rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*dark grey bars*). For both sides, $^{*}P<.05$ vs. ovx controls; $^{**}P<.005$ vs. ovx controls; $^{+}P<.05$ vs. aged controls, $^{++}P<.005$ vs. ovx controls.



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evidence, in ovariectomized rats estrogen deprivation induces a decrease in β-endorphin at central and peripheral levels (13, 19, 24, 25). Aging might be associated with a reduction in brain β-endorphin production and storage, either directly by acting on opiatergic neurons, or indirectly through the derangement of the neuroendocrine systems responsible for opiatergic neurone control. However, it is unknown whether these effects are the consequences of a long-term estrogen deprivation or of central senescence processes. The ovariectomy-induced β-endorphin decrease varies in the different tissues examined, in agreement with previous data (19, 24, 25). This is probably related to the different sensitivity to estrogens of the examined areas or to a different expression of estrogen receptor subtypes (α and β) (34–36). In aged animals, the organ-specific differences in the degree of β-endorphin reduction are even more evident than in young castrated animals, thus suggesting that aging might play a relevant role in modulating the activity of opiatergic neurons.

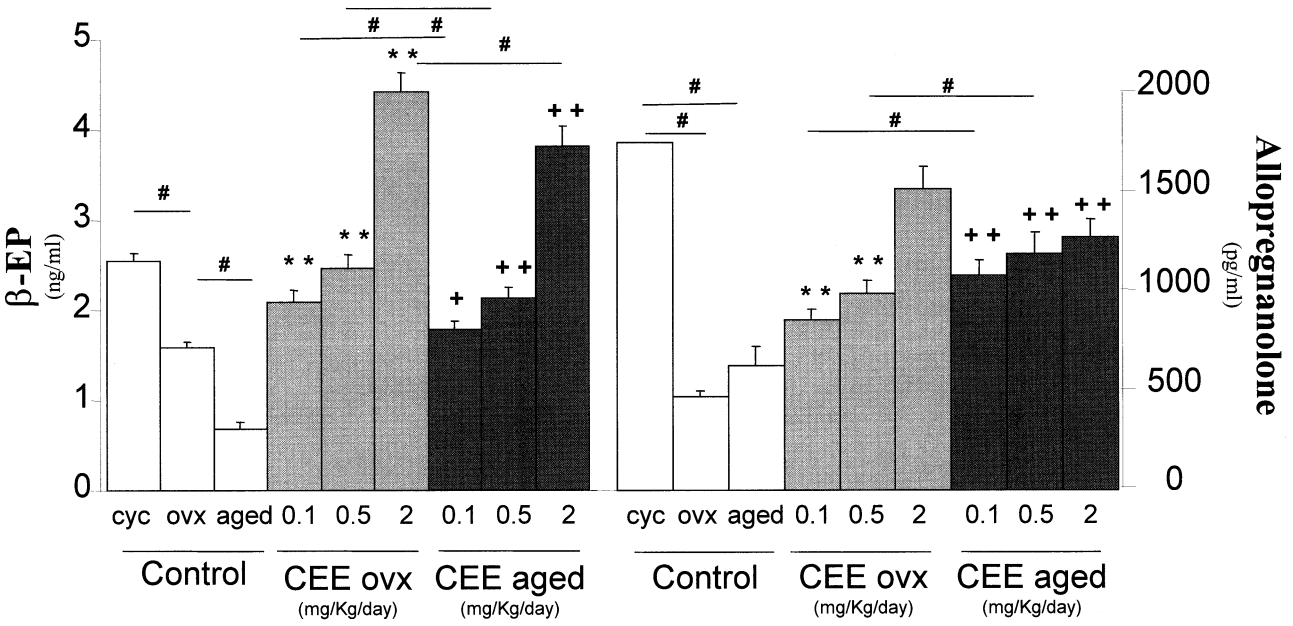
The positive effects of estrogens on β-endorphin levels are confirmed by the fact that CEE administration determines a dose-dependent increase in β-endorphin in all tissues and restores values similar to those of young cycling animals. In young ovx rats the response to CEE administration is more evident than in healthy aged animals, possibly owing to either a higher activity and/or density of estrogen receptors or to a higher efficacy of estrogens on the neuroendocrine systems regulating opiatergic neurons.

Regarding the effects of aging on allopregnanolone, the present study demonstrates a relevant decrease in the brain and serum and a significant increase in the adrenal glands. Differently from β-endorphin, no significant differences in allopregnanolone levels were observed between aged and ovx animals. The mechanisms underlying the effects of aging and of estrogenic deprivation on the central and adrenal allopregnanolone biosynthetic pathways are still unclear.

The neuroprotective action of estrogens within the brain seems to be related to multiple mechanisms. The effect of the topic administration of estrogens on several brain areas was demonstrated in experimental studies in aged animals. Estrogens modulate neuronal activity by stimulating the growth of dendritic spines, synaptic junction formation, and neurotransmission (1–4, 37). Estrogen administration in aged fe-

FIGURE 5

Plasma β-endorphin (β-EP) and serum allopregnanolone levels in cycling, ovx, and aged female rats. Each group consisted of eight rats. *Left:* Beta-endorphin levels in placebo-treated (control) cycling, ovx, or aged rats (*white bars*), in ovx rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*light grey bars*), and in aged rats treated with CEE (0.1, 0.5, 2 mg/kg/day) (*dark grey bars*). *Right:* Allopregnanolone levels in placebo-treated (control) cycling, ovx, or aged rats (*white bars*), in ovx rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*light grey bars*), and in aged rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (*dark grey bars*). For both sides, $^{*}P<.05$ vs. ovx controls; $^{**}P<.005$ vs. ovx controls; $^{+}P<.05$ vs. aged controls, $^{++}P<.005$ vs. ovx controls.



Genazzani. CEE effect on CNS in young and aged rats. Fertil Steril 2004.

male rats allows hypothalamic arcuate nucleus neurons to maintain enough plasticity to react to deafferentation (38) and determines an enlargement of the medial preoptic area, anterior area, and arcuate nucleus of the hypothalamus (39). A further mechanism of estrogen-related neuroprotection is the genomic effect on neurotransmitter biosynthetic pathways. Estrogens enhance the synthesis of acetylcholine, the principal neurotrasmitter regulating memory and learning, by increasing the synthesis of choline acetyltransferase (40, 41).

In addition to the aforementioned mechanisms, sex steroid hormones also modulate β-endorphin synthesis and release via genomic regulation of opiatergic neurons. Previous studies have shown that the influence of opioid peptides on the reproductive axis is altered in aged female rats, in association with the loss of estrous cyclicity (42). Fewer data are available regarding the mechanisms of estrogenic modulation of allopregnanolone synthesis. Estrogens might act directly on the enzymes involved in the allopregnanolone biosynthetic pathway, by modulating 5α-reductase activity in the brain and in the adrenals (43, 44) or by increasing 3α-hydroxysteroid oxydoreductase activity in rat brain (45).

The administration of CEE in aged and ovx rats determines a dose-dependent increase in allopregnanolone levels, restoring values similar to those of cycling animals in most tissues. Whereas β-endorphin levels are uniformly higher in ovx vs. aged rats after CEE administration, the changes in allopregnanolone levels show a more complex pattern. In the hippocampus allopregnanolone response to CEE is similar in ovx and aged rats, whereas in the hypothalamus the increase is higher in ovx animals, and the opposite occurs in the other brain tissues and in serum. Further investigations are necessary to understand the reasons for this tissue-specific allopregnanolone response to estrogenic treatment.

In conclusion, aging negatively affects the synthesis and release of β-endorphin and allopregnanolone in several brain areas and at the peripheral level. The effects of aging are comparable to those of ovariectomy for allopregnanolone, whereas for β-endorphin the reduction is more marked with aging than with ovariectomy. Treatment with CEE in both ovx and aged animals counteracts the changes in allopregnanolone and β-endorphin in a dose-dependent manner, restoring them to levels similar to those of cycling animals or even higher.

The restoration of allopregnanolone and β-endorphin content after estrogen administration in both hypoestrogenic animal models suggests that these compounds might play a role as neuroendocrine mediators of the effects of estrogens on the brain. Further studies are necessary to investigate whether the beneficial effects on verbal memory, attention, and cognition observed in postmenopausal women treated with estrogen replacement therapy are in part related to the positive effects of estrogens on neurosteroids and opioid peptides at the central level.

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