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Effect of estetrol on Beta-Endorphin level in female rats

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ABSTRACT

Introduction: Estetrol (E4), a naturally occurring estrogen produced exclusively by human fetal liver, is currently being evaluated for potential use in contraception and menopausal care in humans. The present study was designed to profile E4 effects on the central nervous system, to assess the *in vivo* effects of E4 administration on Beta-Endorphin (β-END) release in specific brain structures and to evaluate whether E4 has synergic or antagonistic effects on estradiol-mediated β-END synthesis.

Experimental: Intact female adult rats received different doses of E4 and ovariectomized (OVX) rats received different doses of E4 or E2V or combinations of both drugs. The concentrations of β-END were assessed in the frontal and parietal cortex, hippocampus, hypothalamus, neurointermediate lobe, anterior pituitary and plasma.

Results: E4 at the dose of 1 mg/kg/day did not alter β-END content in most brain areas, as well as, plasma levels of intact animals E4 administered at a dose of 5 mg/kg/day decreased β-END content in the hippocampus, hypothalamus, and in the neurointermediate lobe, as well as, plasma levels, compared to intact animals receiving vehicle. E4 increased β-END values in the frontal cortex, but not in the plasma, following the administration of 1 mg/kg/day in OVX rats, whereas treatment with 5 mg/kg/day in OVX rats induced a significant increase in β-END levels in most brain areas and in the plasma. However, in the presence of estradiol, E4 showed an estrogen-antagonistic effect in selected brain structures at the dose of 5 mg/kg/day and in plasma levels of β-END at the dose of 1 mg/kg/day and 5 mg/kg/day.

Conclusion: In OVX rats, E4 increases CNS and peripheral levels of β-END, behaving as a weak estrogen-agonist. The antagonistic effect observed after combined estradiol and E4 administration further profiles E4 as a natural SERM.

1. Introduction

The hormone estetrol (estra-1,3,5(10)-trien-3,15α-16α,17β-tetrol), or E4, is naturally produced from E2 and E3, via 15α- and 16α-hydroxylase, by the human fetal liver during pregnancy. The concentration of E4 increases during pregnancy in both the mother and the fetus, disappearing completely from circulation after delivery [1–3].

E4 has a moderate affinity for ERα and ERβ receptors, with a four- to five-fold preference for the ERα. E4 is highly selective for estrogen receptors and binding to glucocorticoid, testosterone or progesterone receptors was only 11–15% [4]. Therefore, E4 may be envisioned for use in contraception and menopausal care [2].

In well-validated and predictive rat models, E4 acts as a weak estrogen agonist in the bone, myometrium, vaginal epithelium and in the endometrium of ovariectomized (OVX) rats at dosages ranging from 1 to 3 mg/kg/day after at least one week of treatment [5,6]. Similarly, twice-daily administration of E4 at a dosage of 3 mg/kg/day inhibits ovulation in fertile rats [7].

In rats treated with DMBA (7,12 dimethylbenz(a)-anthracene), E4 prevents the growth of mammary tumors in dose-dependent fashion. Additionally, E4 shows estrogen-antagonistic effects in the breast in the presence of estradiol, adding further evidence to its definition as a natural selective estrogen receptor modulator (natural SERM) [8].

In an experimental rat model of menopausal vasomotor symptoms, it has been demonstrated that E4 is effective in preventing body temperature increases. E4 showed a dose-dependent effect in diminishing hot flushes using dosages that are 10-fold less potent than ethinylestradiol, implying E4 estrogen agonist affect in the central nervous system (CNS) [9].

The endogenous opioid system has been implicated in a number of CNS functions which may be altered in various pathological states [10]. Endogenous opioids modulate responses to stress,

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learning, emotional regulation, sexual arousal, memory acquisition and thermoregulation [11]. Beta-Endorphin (β-END) is the most studied endogenous opiate. It has been shown that, in women with premenstrual syndrome and in menopause, plasma β-END is decreased in comparison to fertile, asymptomatic, women, which may suggest a role of the opioidergic system in the development of mood symptoms [12,13]. Sex steroids influence the endogenous opioid system in both sexes [14,15]. Estradiol and combinations of estradiol with synthetic progestins, currently used in combined contraceptives and hormonal replacement therapy, affect differentially the CNS concentration of β-END, suggesting the presence of specific pharmacological effects on opioid release [16–18].

The present study was designed to further profile E4 effects on the CNS and evaluate the *in vivo* effect of E4 on β–END synthesis in specific brain structures and to compare β-END levels to those after estradiol valerate (E2V) treatment. The objectives of the present study were (a) to determine the effect of different doses of E4 administration on β-END levels in intact and ovariectomized rats (b) to compare the effect of E4 administration on the levels of β-END following the administration of E2V in OVX rats in order to define its synergic or antagonistic effect on E2V-mediated β-END synthesis.

2. Experimental

2.1. Animals

Three-month-old adult female Wistar rats weighing between 200 and 250 g were obtained from Charles River Laboratories (Calco, LC, Italy). The rats were housed two per cage and maintained in standard conditions at 22 ± 1 °C and 55–60% humidity. A 12-h reversed light/dark cycle was employed to facilitate behavioral testing during the normally active (dark) phase of the cycle. Commercial rat pellets and water were freely available. After a week of adaptation, the rats were bilaterally ovariectomized under ketamine hydrochloride (Ketavet 100®, Farmaceutici Gellini Spa, Italy) plus xylazine hydrochloride (Rompun®, Bayer, Germany) anesthesia using standard surgical procedures. Animal care, maintenance and surgeries were conducted in accordance with Italian law (D.L. n. 116/1992) and European legislation (EEC n. 86/609). The experimental design and procedures were approved by the Bioethical Committee of the University of Pisa.

2.2. Treatments

Treatments were started approximately 3 weeks after ovariectomy to allow for complete recovery from surgery. To test the effect of E4 on intact and OVX animals, rats were divided into eight groups (eight rats in each group) and submitted to the following treatment protocols (performed in parallel):

(1) Intact rats receiving no treatments (vehicle).
(2) Intact rats plus E4 1 mg/kg/day *per os* for 14 days.
(3) Intact rats plus E4 5 mg/kg/day *per os* for 14 days.
(4) OVX rats receiving no treatment (vehicle).

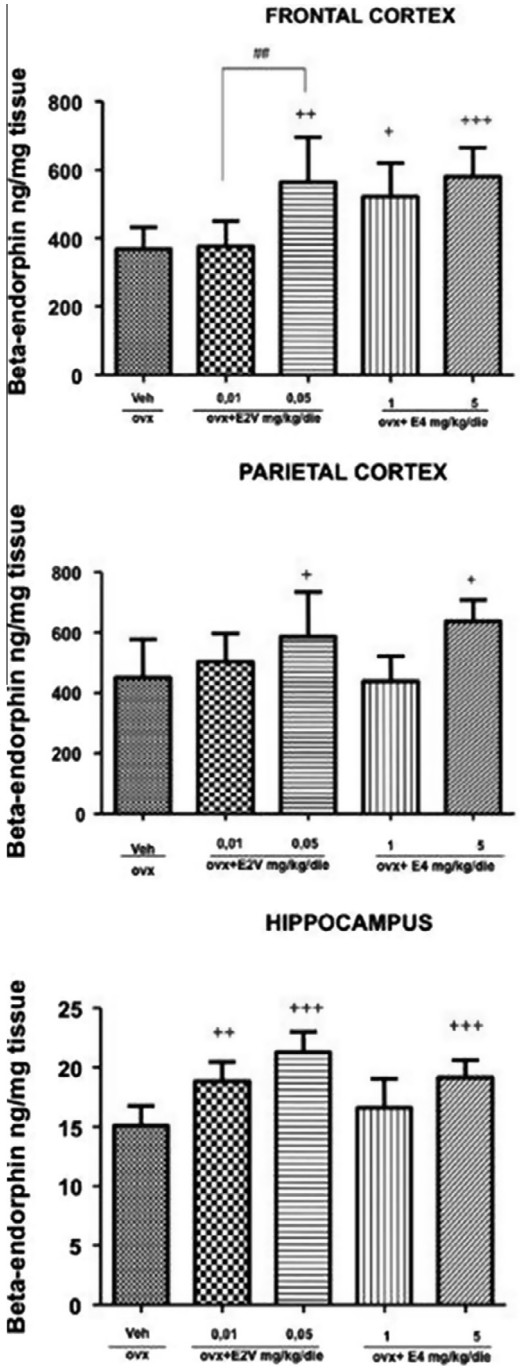

Fig. 1. Effect of E2V or E4 on β-END levels in the frontal and parietal cortex and hippocampus. E2V at dosages of 0.01 or 0.05 mg/kg/day, E4 at a dosage of 1 or 5 mg/kg/day or vehicle (veh) were administered daily to OVX rats for 14 days. β-END concentrations in the frontal and parietal cortex and hippocampus are shown. $^{+}p < 0.05$ vs ovx; $^{++}p < 0.01$ vs ovx; $^{+++}p < 0.001$ vs ovx; $^{\#\#}p < 0.01$ vs lower dose.

Table 1
Beta Endorphin levels in central nervous system (ng/mg tissue) and in the serum (pg/ml) of intact female rats receiving vehicle or E4 at 1 or 5 mg/kg/day.

	Vehicle	E4 1 mg/kg/day	E4 5 mg/kg/day	*p*
Frontal cortex	644.125 ± 113.70	545.75 ± 82.79	591.75 ± 85.41	ns
Parietal cortex	691.75 ± 96.82	622.5 ± 109.69	637.25 ± 67.83	ns
Hippocampus	22.14 ± 3.08	19.16 ± 1.99	16.9 ± 2.49	$p < 0.01$ vehicle + E4 5 mg/kg/day vs vehicle
Hypothalamus	19.66 ± 3.53	17.16 ± 2.88	13.84 ± 2.41	$p < 0.01$ vehicle + E4 5 mg/kg/day vs vehicle
Anterior pituitary	666.375 ± 78.96	646 ± 120.34	647.25 ± 117.76	ns
Neurointermediate lobe	1943 ± 245.71	1503.625 ± 358.43	1337.25 ± 482.09	$p < 0.05$ vehicle + E4 5 mg/kg/day vs vehicle
Plasma (pg/ml)	2.6 ± 0.19	2.5 ± 0.26	2.125 ± 0.35	$p < 0.01$ vehicle + E4 5 mg/kg/day vs vehicle