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Research Article

Dose and Time-Course Evaluation of a Redox-Based Estradiol-Chemical Delivery System for the Brain. II. Pharmacodynamic Responses

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Clinically, brain-enhanced delivery and sustained release of estradiol (E_2) are desirable for effective treatments of menopausal hot flushes and prostatic adenocarcinoma and for fertility regulation. Thus, we conducted studies to determine the dose- and time-dependent effects of a brain-enhanced estradiol-chemical delivery system (E_2-CDS) on anterior pituitary hormones secretion in ovariectomized (OVX) rats. The E_2-CDS has consistently demonstrated preferential retention of its intermediate metabolite (E_2-Q^+), with slow release of E_2 in the brain but rapid clearance from peripheral tissues. Animals received a single iv injection of E_2-CDS at doses of 0.01, 0.1, or 1.0 mg/kg or an E_2 dose of 0.7 mg/kg on day 0. The responses of plasma luteinizing hormone (LH), follicle-stimulating hormone (FSH), growth hormone (GH), and prolactin (PRL) were then evaluated at 1, 7, 14, 21, or 28 days after drug administration. The E_2-CDS caused a dose- and time-dependent suppression of LH and FSH throughout the time course studied. The maximum LH and FSH reduction occurred at 7 days postinjection. Plasma LH and FSH were significantly suppressed by 86 and 58% on day 7, respectively, and were suppressed by 35% (LH) or were at preinjection levels (FSH) at 28 days following the single injection of a 1.0-mg E_2-CDS dose. An equimolar E_2 dose suppressed LH and FSH by only 29 and 20% on day 7, respectively which were not significantly different from time 0 values. Plasma PRL increased significantly on day 14 with the 1.0-mg E_2-CDS dose but levels returned to preinjection values by 28 days after drug administration. Lower doses of the E_2-CDS did not affect PRL concentrations. Plasma GH concentrations were not altered in response to the E_2-CDS at any dose or time. Also, anterior pituitary and uterine weights increased in a dose- and time-dependent manner in response to E_2-CDS administration. Collectively, these data demonstrate that the E_2-CDS effects on gonadotropins suppression are dose and time dependent and this duration of suppression is consistent with the long half-lives of the E_2-CDS metabolites in the brain.

KEY WORDS: estradiol; estradiol delivery system; blood–brain barrier; pharmacodynamics; gonadotropins.

INTRODUCTION

Physiologically, estrogen hormones exert two modes of action on the brain: (i) during the critical period of fetal/neonatal life, they affect permanently some features of the brain structure and function which result in neuronal differentiation; and (ii) during the adult life, these hormones exert their effects in a modulatory, reversible mode that influence a myriad of adult brain functions. The latter central action of estrogens, in particular estradiol (E_2), is of significant therapeutic interest due to the existence of clinical conditions which are influenced by brain estrogens. For instance, the brain is the primary locus where E_2 exerts its effects to inhibit the secretion of luteinizing hormone-releasing hormone (LHRH) and hence of luteinizing hormone (LH) and gonadal steroids (1–3). As such, estrogens are used therapeutically for fertility regulation and, also, to reduce the growth of peripheral androgen-dependent tumors such as prostatic adenocarcinoma (4,5). Additionally, estrogens are believed to act centrally to stimulate male and female sexual behaviors (6) and may have influences on mood (7,8) and cognitive function (9,10).

Furthermore, the endogenous production of estrogens decline at menopause, which then leads to a number of brain-mediated estrogen withdrawal symptoms (11,12). Hot flushes and psychological changes associated with the menopause are believed to result from brain deprivation of estrogens (11,12), and the pharmacotherapy of these symptoms is estrogen replacement (13,14).

Given the aforementioned evidence for the central ac-

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tions of estrogen and hence the therapeutic potential of a brain-estrogen delivery, we evaluated the pharmacodynamic responses of an estradiol-chemical delivery system (E_2-CDS) for the brain.

The E_2-CDS is a redox-based delivery system and the mechanism of its brain-enhanced delivery is based on an interconvertible dihydropyridine⇌pyridinium salt carrier (15). The mechanism leading to brain-enhanced delivery and sustained release of E_2 requires multiple, facile chemical conversion of E_2-CDS, including oxidation of E_2-CDS to the corresponding quaternary pyridinium salt (E_2-Q^+), which provides the basis of locking the molecule in the brain, and hydrolysis of E_2-Q^+ to E_2.

Recently, we conducted a series of studies to investigate the tissue distributions of both E_2-Q^+ and E_2 (two metabolites of the E_2-CDS) in intact male (16) as well as in ovariectomized (OVX) female rats (17). The E_2-CDS has consistently demonstrated its predictive pharmacokinetic behaviors, that is, the preferential retention of E_2-Q^+ and E_2 in the brain, with an apparent $t_{1/2}$ = 8–9 days, while it simultaneously accelerates the elimination of these metabolites from the periphery. On the basis of these pharmacokinetic behaviors, the E_2-CDS is expected to exhibit pharmacodynamic responses with a long duration of action following a single administration.

Our previous pharmacological studies with the E_2-CDS showed prolonged pharmacodynamic effects following a single iv injection including LH suppression in castrated male rats for greater than 24 days, body weight suppression for 36 days, and stimulation of copulatory behaviors in castrated male rats for 36 days after doses of 1 to 3 mg E_2-CDS/kg (18–22).

The present study was undertaken to determine whether the long half-lives and the magnitude of E_2-CDS metabolites in brain tissue correlate with the duration of pharmacodynamic effects. More specifically, our objectives were (1) to assess the dose- and time-dependent effects of the E_2-CDS on brain-mediated responses; (2) to compare E_2-CDS with equimolar E_2; and (3) to correlate the half-lives of the E_2-CDS with the duration of pharmacodynamic effects mediated by E_2. Our evaluation of the distribution of E_2-Q^+ and E_2 in the same animals used for the present pharmacodynamic evaluations is presented in the preceding paper (17).

MATERIALS AND METHODS

Animals and Drug Treatment

All the samples assayed in this series were obtained from the animals used and described in the preceding report (17); this study presents further data on evaluation of the pharmacodynamic responses of E_2-CDS. Briefly, rats were ovariectomized (OVX) and, 2 weeks later, were administered a single iv injection (tail vein) of the E_2-CDS at doses of 0 (HPCD), 0.01, 0.1, or 1.0 mg/kg body weight or E_2 at a dose of 0.7 mg/kg (equimolar to 1.0 mg E_2-CDS dose). Animals (seven per group) were then killed by decapitation 1, 7, 14, 21, or 28 days after the drug administration and plasma and tissue samples were collected for later analysis.

Plasma Hormone Analysis

Plasma luteinizing hormone (LH), follicle-stimulating hormone (FSH), growth hormone (GH), and prolactin (PRL) concentrations were measured in duplicate by radioimmunoassay (RIA) using NIADDK kits provided by the Pituitary Hormone Distribution Program. Plasma LH, FSH and GH values are expressed as nanograms per milliliter of either the LH-RP-2, the FSH-RP-2, or the GH-RP-2 reference standard, respectively, and PRL values are expressed as nanograms per milliliter of the PRL-RP-3 standard. The intraassay coefficients of variation were 4.67, 5.02, 4.05, and 4.96% for LH, FSH, GH, and PRL assays, respectively. All the samples for each hormone were assayed in a single run.

Statistics

The significance of interaction between factors (time and dose) was determined by two-way analysis of variance (ANOVA). The significance of differences among mean values at each dose level was determined over time by one-way ANOVA and Dunnett's test, while the significance of differences among mean values of three dose levels (at each time point) was determined by one-way ANOVA and Scheffe F test (23). The level of probability for all tests was $P < 0.05$.

RESULTS

The E_2-CDS caused a dose- and time-dependent suppression of plasma LH throughout the time course studied (Fig. 1, upper panel). The maximum LH reduction occurred at 7 days postinjection. At this time, LH was suppressed by 21, 46, and 86% relative to HPCD control at doses of 0.01, 0.1, and 1.0 mg E_2-CDS/kg, respectively (Fig. 1). The plasma LH concentrations in animals treated with 1.0 mg E_2-CDS were significantly reduced by 56, 86, 72, and 56% at 1, 7, 14, or 21 days, respectively, and remained suppressed by greater than 35% at 28 days after drug administration. In contrast, equimolar E_2 dose (0.7 mg/kg) caused a transient reduction in LH concentrations of 27% on day 1 and 24% on day 7, which were not significantly different from time 0 values (Fig. 1, upper panel).

Similarly, the E_2-CDS caused a dose- and time-dependent suppression of plasma FSH throughout the time course studied (Fig. 1, lower panel). The maximum FSH reduction occurred at 7 days postinjection. FSH was suppressed by 14, 28, and 58% relative to control at doses of 0.01, 0.1, and 1.0 mg E_2-CDS/kg, respectively, on day 7 (Fig. 1). The plasma FSH concentrations in animals treated with 1.0 mg E_2-CDS were significantly reduced by 37, 58, and 20% at 1, 7, or 14 days, respectively, and were reduced by 7% (day 21) or were at preinjection levels by 28 days after drug administration. In contrast, an equimolar E_2 dose reduced plasma FSH by 27% at day 1 and 19% at day 7 (Fig. 1, lower panel).

Plasma concentrations of LH and FSH in animals treated with lower doses of the E_2-CDS (0.01 and 0.1 mg/kg) began to increase gradually after 7 days of drug administration (Fig. 1).

Plasma PRL concentrations in animals treated with 1.0 mg E_2-CDS dose were increased 4-, 8-, 13-, and 8-fold at 1, 7, 14, and 21 days, respectively, or were at preinjection levels by 28 days after drug administration (Fig. 2, upper panel). Lower doses of the E_2-CDS did not affect PRL concentrations. In contrast, the 0.7-mg/kg dose of E_2 increased plasma

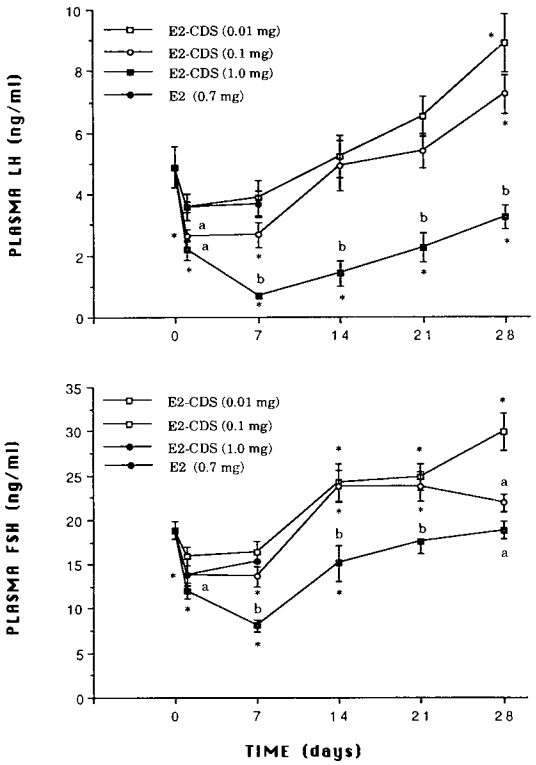

Fig. 1. Dose- and time-dependent effects of the E_2-CDS on the plasma LH responses (upper panel) and FSH responses (lower panel) in OVX rats. Animals received a single iv injection of the E_2-CDS on day 0 at doses of 0.01 (□), 0.1 (○), and 1.0 (■) mg/kg. Also, the responses to an E_2 dose of 0.7 mg/kg (●), equimolar to the 1.0-mg/kg dose of E_2-CDS, is shown for days 1 and 7. Represented are means ± SE for n = 7 rats per group per sampling time. The symbols indicate statistical differences as follows: (*) different from vehicle group (day 0); (a) different from 0.01 mg/kg; and (b) different from both 0.01 and 0.1 mg/kg.

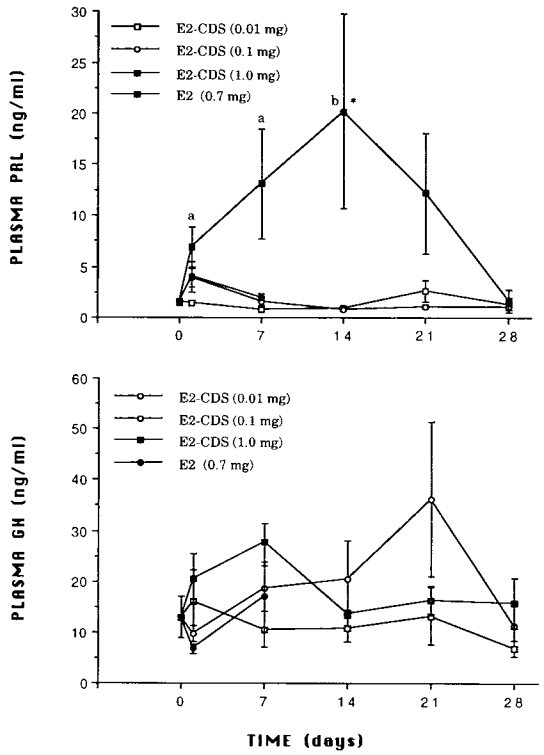

Fig. 2. Dose- and time-dependent effects of the E_2-CDS on the plasma PRL responses (upper panel) and GH responses (lower panel) in OVX rats. Animals received a single iv injection of the E_2-CDS on day 0 at doses of 0.01 (□), 0.1 (○) and 1.0 (■) mg/kg. Also, the responses to an E_2 dose of 0.7 mg/kg (●), equimolar to the 1.0-mg/kg dose of E_2-CDS, is shown for days 1 and 7. Represented are means ± SE for n = 7 rats per group per sampling time. The symbols indicate statistical differences as follows: (*) different from vehicle group (day 0); (a) different from 0.01 mg/kg; and (b) different from both 0.01 and 0.1 mg/kg.

PRL concentrations threefold on day 1 and PRL returned to preinjection levels by day 7 after drug administration (Fig. 2).

Plasma GH concentrations were not altered in response to E_2 or E_2-CDS at any dose or time tested (Fig. 2, lower panel).

Anterior pituitary weights increased in a dose- and time-dependent manner in response to E_2-CDS administration (Table I). With the lower doses of the E_2-CDS (0.01 and 0.1 mg/kg), pituitary weights were slightly increased (22 to 32%) over control-group weights by 14 days postinjection, but they returned to control levels by day 21. However, the highest dose of the E_2-CDS increased pituitary weights significantly from day 7 to day 28 relative to weights at time 0 and following treatment with lower doses of E_2-CDS. The maximum pituitary gland stimulation occurred at 14 days postinjection and then weights began to decrease but remained elevated at 28 days after the drug administration (Table I).

Similarly, uterine weights showed a dose- and time-dependent increase in response to E_2-CDS administration (Table I). Uterine weights were increased by 20, 54, or 82% on day 1 following treatment with the E_2-CDS at 0.01-, 0.1-, and 1.0-mg/kg doses, respectively. With the highest dose of the E_2-CDS (1.0 mg/kg), uterine weights were significantly increased by about threefold on day 7, then weights began to decrease but remained elevated at 28 days after the drug administration (Table I). It should be noted that even at the highest dose (1.0 mg E_2-CDS/kg), uterine weights were less than those typically observed in gonad-intact rats (500–625 mg).

An equivalent increase in anterior pituitary as well as uterine weights was observed with E_2 (0.7 mg/kg) compared to the 1.0-mg E_2-CDS dose on day 1. However, by day 7 the effects of equimolar E_2 were equivalent to the lowest dose of E_2-CDS (0.01 mg/kg).

DISCUSSION

The results of this study demonstrated that the E_2-CDS causes dose- and time-dependent suppression of gonadotropin (LH and FSH) secretion in OVX rats and that maximum reductions in plasma LH and FSH concentrations occur 7 days after the E_2-CDS administration. The time course of gonadotropin suppression in OVX rats is comparable to that previously observed for E_2-CDS effects on other parameters and in other animal models. We have reported long-term suppression of LH in castrated male rats (18), suppression of testosterone secretion for 2–3 weeks (19), stimulation of

Table I. Dose- and Time-Dependent Effects of the E_2-CDS on Peripheral Tissue Weights in OVX Rats[a]

Tissue	Drug	Dose (mg/kg)	Days after treatment				
			0	1	7	14	21	28
Anterior pituitary (mg)	HPCD	—	11.8 ±0.5					
	E_2	0.7		13.5 ±0.7	12.8 ±0.7	ND	ND	ND
	E_2-CDS	0.01		12.0 ±0.8	13.1 ±0.5	14.4 ±1.5	11.9 ±0.8	13.0 ±0.6
	E_2-CDS	0.1		12.8 ±0.9	15.6* ±0.9	14.9* ±1.5	13.1 ±0.5	12.6 ±0.6
	E_2-CDS	1.0		13.3 ±0.9	19.0*,*** ±1.0	20.7*,*** ±1.3	16.2*,*** ±1.0	15.0*,**** ±0.6
Uterus (mg)	HPCD	—	154.2 ±8.6					
	E_2	0.7		261.4* ±13.0	182.9 ±24.0	ND	ND	ND
	E_2-CDS	0.01		183.8 ±12.3	179.1 ±29.0	196.4 ±42.0	121.5 ±7.0	107.8 ±8.0
	E_2-CDS	0.1		237.7* ±12.0	234.4* ±27.0	210.1* ±33.0	161.1 ±8.0	128.4 ±5.0
	E_2-CDS	1.0		281.4*,** ±20.0	427.0*,*** ±31.0	372.9*,*** ±28.0	267.4*,*** ±31.0	235.4*,*** ±16.0

[a] Values are the mean tissue weights ± SE. ND, not determined.
* Different from time 0 values.
** Different from 0.01-mg/kg dose.
*** Different from 0.01- and 0.1-mg/kg doses.
**** Different from 0.1-mg/kg dose.

masculine sexual behavior in castrated male rats for 28 days (20), reduction in weight of androgen-responsive tissue (21), and body weight alterations for 36 days (22) following a single iv administration of the E_2-CDS. Sarkar *et al.* have reported suspension of estrous cycles for 30 days following E_2-CDS treatment (24). These prolonged pharmacological effects further support the idea that the intermediate metabolite of the E_2-CDS, E_2-Q^+, is "locked" behind the BBB and there serves as a brain depot for E_2 (15–17). From this store of E_2-Q^+, E_2 is then slowly released through nonspecific hydrolysis of the carrier, resulting in sustained brain exposure to E_2.

Since 17-substituted estrogens, like the E_2-CDS and E_2-Q^+, do not effectively bind to E_2 receptors (25), they are not likely to exhibit estrogenic activity. It is reasonable to believe that neither the E_2-CDS nor the E_2-Q^+ formed in the brain account for the prolonged pharmacological effects of this delivery system. Rather locally released E_2 in the brain, in particular the hypothalamus, would appear to account for the sustained suppression of the gonadotropins secretion.

Our previous evaluation of tissue distribution of the E_2-CDS in male rats (16) and the more detailed dose–response and time-course evaluation of the E_2-CDS distribution in OVX rats (17) revealed that (i) E_2-Q^+, the quaternary form of E_2-CDS, as well as E_2 persists in the brain with $t_{1/2}$ = 8–9 days and (ii) the same metabolites are rapidly eliminated from the peripheral tissues. These, together with the absence of a physiologically significant elevation of plasma E_2 concentrations from 7 to 28 days after the E_2-CDS administration (17), provide strong evidence for the local action of E_2 in the brain, presumably on hypothalamic luteinizing hormone-releasing hormone (LHRH)-containing neurons (24).

The synthesis and secretion of gonadotropins from the anterior pituitary are regulated by several neuronal (26) and hormonal (1,3) factors including the hypothalamic decapeptide, luteinizing hormone-releasing hormone (LHRH), and the action of E_2 in both a positive and a negative feedback mode at the hypothalamus as well as the anterior pituitary. The evaluation of the effects of E_2-CDS on LHRH neuronal activity (i.e., LHRH release) showed that portal blood concentrations of LHRH were significantly reduced for more than 16 days following the treatment (24). The reduced LHRH secretion was in contrast to the increased hypothalamic LHRH concentrations, suggesting that the inhibition of release resulted in a tissue buildup of the decapeptide. Furthermore, chronic exposure to E_2 has no significant effects on anterior pituitary responsiveness to LHRH (27), indicating that the prolonged inhibitory effects of E_2-CDS on LH and FSH are due primarily to sustained suppression of LHRH secretion from the hypothalamus.

When the dynamics of the E_2-CDS effects were compared with that of an equimolar dose of E_2, the E_2-CDS showed 100-fold greater effectiveness in the magnitude of inhibition of plasma LH and FSH. In other words, when compared on a molar basis, the magnitude of E_2 effects was equivalent to that of the E_2-CDS with a 100-fold lower dose (Fig. 1). This marked increase in effectiveness and the prolonged duration of the E_2-CDS effects on LH and FSH secretion are most likely due to "lock-in" of the E_2-Q^+ with subsequent slow release of E_2 in the brain.

When the kinetic behaviors of E_2-CDS and E_2 were compared on molar basis, the E_2-CDS (1.0 mg/kg) produced E_2 concentrations in brain tissue which were 81- and 182-fold greater than after an equimolar E_2 (0.7 mg/kg) treatment at 1 and 7 days postinjection, respectively (17). Therefore, it seems more reasonable to suggest that following the E_2-CDS administration, the brain E_2 is continuously produced, and as such the steady-state brain concentrations of E_2 is dependent on its rate of production from the E_2-Q^+ and its rate of elimination from the brain by either local metabolism or its redistribution down a concentration gradient into the general circulation.

We observed a significant elevation in plasma PRL in response to the highest dose of E_2-CDS (1.0 mg/kg), whereas lower doses had no significant effect on plasma PRL concentrations. It appears, then, that elevations in plasma PRL correlate with the administration of E_2-CDS at doses which result in the transient elevation of plasma E_2 levels but not at doses at which plasma E_2 remains low (17). This apparent stimulation of PRL production by the E_2-CDS would appear to be due to the well-described actions of E_2 on the anterior pituitary lactotrophes (28). However, the lack of a positive temporal correlation between plasma PRL (present study) and plasma E_2 (17) levels suggests the possibility that E_2 released in the brain might be responsible for a direct stimulation of the anterior pituitary. This can be explained by the anatomical relationship between the hypothalamus and the anterior pituitary gland. E_2 released from the E_2-Q^+, or the E_2-Q^+ itself, which is "locked" into the brain, could be delivered directly to the anterior pituitary by the capillary plexus of the hypophyseal portal system (29). These capillaries in the median eminence lack features of other brain capillaries and hence are not part of the BBB (29).

The E_2-CDS had no significant effects on the mean plasma GH concentrations over the 28-day time course, at any of the three doses examined. However, a careful analysis of the effects of E_2-CDS on pulsatile GH secretion (30) revealed that while mean GH levels are not changed, baseline GH values were elevated and GH pulse amplitudes were moderately reduced at 7 days after E_2-CDS administration.

The marked increases in anterior pituitary weights of OVX rats treated with the E_2-CDS appear to be due to the direct effects of E_2 on the pituitary gland. These effects of E_2 appear to be exerted on the lactotroph population of the anterior pituitary (28,31). E_2 is well known to stimulate PRL secretion and to induce hyperplasia of lactotrophs (28,31). As indicated above, brain E_2 likely reaches the anterior pituitary gland, through the redistribution of the steroid down the marked concentration gradient from the brain to the pituitary gland (29). It should be noted, however, that the effects of E_2-CDS on pituitary weight are dependent upon the OVX condition of the rats. In gonad-intact rats, E_2-CDS does not alter anterior pituitary weight (unpublished observation).

The uterotrophic effects of E_2-CDS were also dose and time dependent. This effect of E_2-CDS likely relates to the extreme sensitivity of OVX rats to circulating estrogens (32). Thus, even modest elevations in plasma E_2 following administration of E_2 or E_2-CDS (17) result in stimulation of uterine tissue. Further, like the anterior pituitary response to E_2-CDS, the uterus of gonad-intake rats is unresponsive to the delivery system (33). Finally, it should be noted that the uterine weights observed following E_2-CDS were considerably lower than the 500- to 625-mg weights normally seen in gonad-intact rats (33).

In conclusion, the prolonged effects of the E_2-CDS on gonadotropins suppression were dose and time dependent, and the durations of these responses are consistent with the long half-lives of the E_2-CDS metabolites in the brain. These results further support the view that the E_2-CDS may be potentially useful in fertility regulation, in the treatment of brain E_2 deficiencies (i.e., vasomotor hot flushes), and in the treatment of androgen-dependent diseases (i.e., prostatic cancer) by virtue of suppressing androgen hormones. In comparison to the currently used estrogenic products, the E_2-CDS should achieve the sustained stimulation of brain E_2 receptors at lower doses or with less frequent dosing.

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