17β-Estradiol Stimulates Ascorbic Acid and LHRH Release from the Medial Basal Hypothalamus in Adult Male Rats

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In the present investigation, 17β-estradiol (E_2) and tamoxifen, an antiestrogen, were evaluated for their effects on the release of ascorbic acid (AA) and luteinizing hormone-releasing hormone (LHRH). Medial basal hypothalami (MBH) from adult male rats were incubated with graded concentrations of E_2 (10^{-9} to 10^{-6} *M*) or a combination of E_2 (10^{-7} *M*) and tamoxifen (10^{-7} and 10^{-6} *M*) in 0.5 ml of Krebs Ringer bicarbonate buffer for 1 hr. AA and LHRH in the incubation medium were measured by high-performance liquid chromatography and radioimmunoassay, respectively. E_2 significantly elevated both AA and LHRH release and the minimal effective dose was 10^{-7} *M*. A combination of E_2 (10^{-7} *M*) and tamoxifen (10^{-6} *M*) totally blocked E_2-induced AA and LHRH release. The stimulatory effect of E_2 was also suppressed in the presence of N^G-monomethyl-L-arginine, a competitive inhibitor of nitric oxide synthase (NOS), illustrating that the release is mediated by nitric oxide (NO). To further characterize the role of NO, the tissues were incubated with E_2 or a combination of E_2 + (6 anilino-5, 8-quinolinedione) LY 83583 (10^{-6} and 10^{-5} *M*), an inhibitor of NOS. LY 83583 was effective in suppressing E_2-induced AA and LHRH release, demonstrating that the effect was mediated by cyclic GMP. Incubation of the tissues with E_2 or a combination of E_2 + 1H-[1,2,4] oxadiazolo [4,3-a] quinoxalin-1-one (O.D.Q.) (10^{-5} and 10^{-4} *M*), a specific inhibitor of soluble guanylyl cyclase failed to alter AA release but significantly suppressed LHRH release. The role of a prostaglandin synthesis blocker in E_2-induced AA and LHRH release was tested by incubating the tissues with E_2 or a combination of E_2 + indomethacin (1.8×10^{-7} or 1.8×10^{-6} *M*). Indomethacin produced a significant decrease in E_2-induced AA and LHRH release, suggesting that the release process required prostaglandins as an intracellular mediator. In conclusion, E_2 stimulated both AA and LHRH release and the effect was mediated by NO and prostaglandins. Exp Biol Med 229:926–934, 2004

Key words: tamoxifen; LY 83583 (NOS inhibitor); O.D.Q (GC inhibitor); indomethacin

Introduction

17β-Estradiol (E_2), the most biologically active form of estrogen, and estrone, are the major natural estrogens produced by the ovary (1, 2). Although E_2 is the major female sex hormone, its presence in male vertebrates and its receptors in male reproductive organs have been reported earlier (3, 4). Thus E_2 plays an important role in many different types of tissues affecting both female and male physiology (3–5). Androgens serve as prohormones for estrogens, and conversion of androgens to estrogens involves multiple steps (6–8). The final aromatization step is catalyzed by an aromatase enzyme complex (6–8). This enzyme is present in several tissues such as the brain, adipose tissue, gonads, bone, and placenta (6–8) and enables them to synthesize estrogens from androgens as well as use to estrogen in a paracrine or autocrine fashion (7). Interruption of the aromatase enzyme complex (*Cyp19*) gene in the aromatase-deficient mouse results in depletion of endogenous estrogen, impairment of sexual behavior, and age-dependent disruption of spermatogenesis, emphasizing the important role of estrogen in male reproduction (9). Estrogen also plays an important role in several physiological functions such as skeletal development, and carbohydrate and lipid metabolism (7, 10, 11). In the male, estrogen derived from both testis and extragonadal aromatization of testosterone and androstenedione is the major hormone responsible for pubertal onset of growth, skeletal maturation, and maintenance of bone mass (11). Androgens and estrogens may alter a variety of other activities of the central nervous system, including the performance of spatial tasks, fine motor skills, and verbal memory tests (12–15). Several investigations also support the view that estrogen may affect the symptoms of neurological diseases such as

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Parkinson and Alzheimer diseases, schizophrenia, and may enhance recovery from neurological injury such as stroke (16–18).

Two types of estrogen receptors (ERs), estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) have been isolated and extensively characterized (19, 20). ERα is found in estrogen-responsive tissues such as the pituitary, ovary, uterus, lung, kidney, adrenals, and mammary glands (21–23). A separate form of ERβ has been isolated and detected in tissues such as prostate, ovary, uterus, lung, and various parts of the central and peripheral nervous systems (24, 25). In the brain, ERβ is the major ER in the olfactory lobe, cortex, and cerebellum (24, 25). Coexpression of ERα and ERβ in the hypothalamus and its presence in the same cell has also been demonstrated (26).

Recently, the antioxidant effect of estrogen has been reported and is shown to be much greater than that of ascorbic acid (AA), a potent antioxidant present in much higher concentrations than estrogens in the brain (27–32). Previously, we have shown that AA acts as an inhibitory transmitter in the hypothalamus to inhibit stimulated luteinizing hormone-releasing hormone (LHRH) release by scavenging nitric oxide (NO) from the medial basal hypothalamus (MBH) (33). 17β-Estradiol, AA, and LHRH are essential to gonadal function and reproduction. However, the effect of E_2 on AA release from the hypothalamus is not known. Therefore, the present study was designed to evaluate the effect of E_2 on the release of AA and LHRH from MBH of adult male rats. In addition, the effects of N^G monomethyl amine (NMMA), a competitive inhibitor of nitric oxide synthase (NOS); LY 83583, an NOS inhibitor (34); 1H-[1,2,4] oxadiazolo [4,3-a] quinoxalin-1-one (O.D.Q.), a selective inhibitor of soluble guanylyl cyclase (GC; 35); and indomethacin, a prostaglandin synthesis blocker (36) have been ascertained. The results support a role for NO, cGMP, and prostaglandin in AA and LHRH releasing action of E_2.

Materials and Methods

Animals. Adult male rats of the Sprague-Dawley strain (200–250 g; Holtzman, Madison, WI) were housed two per cage under controlled conditions of temperature (23–25°C) and lighting (lights-on 0500–1700 hrs). The animals had free access to a pellet diet and tap water.

Chemicals. Sodium ascorbate, NMMA, tamoxifen, indomethacin, bacitracin, O.D.Q., and LY 83583 were purchased from Sigma (St. Louis, MO).

***In Vitro* Studies; Incubation of MBH.** Animals were euthanized by decapitation and the brain was exposed by a dorsal incision. MBH were dissected by vertical cuts along the lateral hypothalamic sulci, posterior edge of the optic chiasma, and the anterior edge of the mammillary bodies. A horizontal cut 1 mm from the base separated the island. MBH (8–12 mg) were incubated *in vitro* as previously reported (33). In brief, one MBH/tube was placed in 0.5 ml of Krebs-Ringer bicarbonate (KRB, pH 7.4) buffer supplemented with 20 μ*M* bacitracin in an atmosphere of 95% O_2 and 5% CO_2 in a Dubnoff shaker (50 cycles/min) for a period of 60 mins. Following this preincubation, the tissues were incubated in 0.5 ml KRB or KRB containing various concentrations of E_2 (10^{-9} to 10^{-6} *M*) for 1 hr. In order to assess the role of tamoxifen, an antiestrogen, the tissues were incubated with tamoxifen or a combination of tamoxifen (10^{-7} and 10^{-6} *M*) plus E_2 (10^{-7} *M*) for 1 hr. The tissues were incubated with E_2 (10^{-7} *M*) or a combination of E_2 (10^{-7} *M*) plus NMMA (3×10^{-4} *M*), a competitive inhibitor of nitric oxide synthase (NOS) to study the role of NO in E_2-induced AA and LHRH release. Experiments were also performed with E_2 (10^{-7} *M*) or a combination of E_2 (10^{-7} *M*) plus LY 83583 (10^{-6} and 10^{-5} *M*) or E_2 (10^{-7} *M*) and a combination E_2 (10^{-7} *M*) plus O.D.Q. (10^{-5} and 10^{-4} *M*) to ascertain the role of NO and cGMP in E_2-induced LHRH and AA release. Finally, the tissues were incubated with indomethacin, a prostaglandin synthesis blocker, to evaluate the influence of prostaglandins as an intracellular mediator in E_2-induced AA and LHRH release. At the end of the experiment, the medium was aspirated and medium and tissues were stored at −80°C. Ascorbic acid and LHRH released into the incubation medium were analyzed by high-performance liquid chromatography (HPLC) and radioimmunoassay, respectively.

Chromatography. Isocratic analyses were carried out with Beckman system gold HPLC equipped with a 126-module and diode array detector 168 operating at 254 nm (Beckman Instruments, Fullerton, CA). The separation was carried out on a Bondapack Beckman ultrasphere C18 column (average particle size 5 μm, 25 cm × 4.6 mm). The mobile phase was a buffer consisting of 0.1 *M* sodium dihydrogen phosphate (NaH_2PO_4) and 0.2 m*M* Na_2EDTA adjusted to pH 3.1 with orthophosphoric acid. The buffer was filtered through a 0.45-μm membrane filter (Gelman Sciences, Ann Arbor, MI) and degassed prior to use. The column was maintained at room temperature, and the mobile phase was used at a constant flow rate of 1.0 ml/min.

Preparation of Standard. A sample buffer consisting of 5 mM each of metaphosphoric acid and Na_2EDTA was prepared in HPLC-grade water (V.W.R. Scientific Products, Dallas, TX) and was used for preparing AA standards and MBH homogenates. The sample buffer was previously shown to stabilize AA solution for 3–4 hrs, and all the estimations were completed within this time (37). A standard curve for AA was prepared from a stock solution of 1 mg/ml and was found to be linear from 487.5 to 7800 ng. A standard curve was constructed with every batch of unknown samples. AA in standards, incubation medium, and homogenates were measured using a 507 ASE auto sampler (Beckman Instruments), and samples were used at a volume of 30 μl. Each sample (unknown) was passed

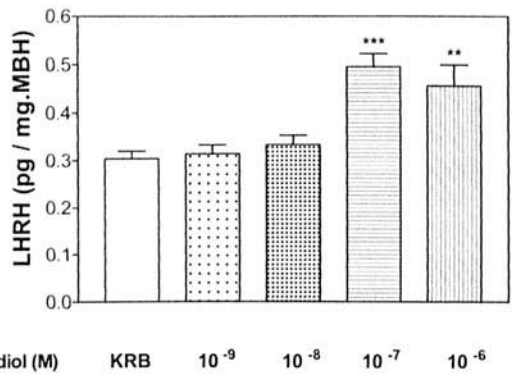

Figure 1. Effect of graded concentrations of 17β-estradiol (E_2) on luteinizing hormone-releasing hormone (LHRH) release from medial basal hypothalamus (MBH) after 1 hr of incubation. $^{**}P < 0.01$ or $^{***}P < 0.001$ vs. Krebs-Ringer bicarbonate (KRB). In this and subsequent figures, the results are the mean ± SEM. There are eight tissues for each group.

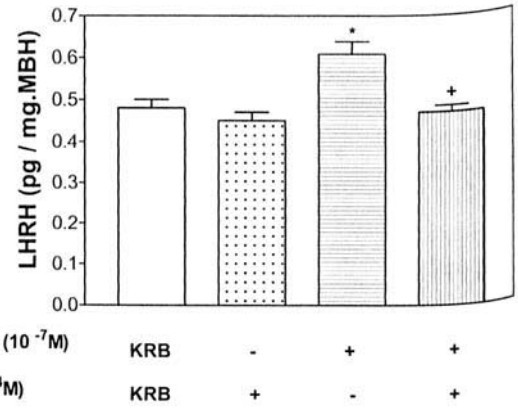

Figure 3. Effect of 17β-estradiol (E_2) or a combination of E_2 and N^G monomethyl amine (NMMA) on luteinizing hormone-releasing hormone (LHRH) release after 1 hr of incubation. $^{*}P < 0.05$ vs. Krebs-Ringer bicarbonate (KRB). $+P < 0.05$ vs. the group treated with E_2. MBH, medial basal hypothalamus.

through syringe filters (Gelman Sciences) before placing it in the vial for counting. A standard calibration plot was obtained for AA concentrations (μg/ml) versus peak area (numerical units on the 126 module).

LHRH Assay. LHRH was assayed as described (38) using a highly specific antibody to LHRH kindly provided by Dr. A. Barnea (University of Texas Southwestern Medical Center, Dallas, TX). The minimal detectable LHRH was 0.2 pg/tube, and the curve was linear up to 100 ng/tube. The inter- and intraassay variations were 5% and 4%, respectively.

Statistics. Results were analyzed by one way analysis of variance or unpaired *t* tests wherever applicable, and $P < 0.05$ was considered significant.

Results

Effect of Graded Concentrations of E_2 on LHRH Release. Incubation of MBH with graded concentrations of E_2 (10^{-9} and 10^{-6} *M*) for 1 hr significantly stimulated LHRH release (Fig. 1). The lowest effective dose was 10^{-7} *M*, and this concentration was used for all other experiments. A similar increase in LHRH release was observed at 10^{-6} *M*.

Influence of E_2 or a Combination of E_2 and Tamoxifen on LHRH Release. In order to assess the role of tamoxifen, an antiestrogen, on E_2-induced LHRH release, the tissues were incubated with either E_2 or a combination of E_2 and tamoxifen (10^{-7} and 10^{-6} *M*) for 1 hr. Tamoxifen at both doses failed to alter basal LHRH release (Fig. 2). A combination of E_2 and tamoxifen (10^{-7} *M*) was ineffective in lowering LHRH release. However, a combination of E_2 and a 10 times higher dose of tamoxifen (10^{-6} *M*) completely blocked E_2-induced LHRH release (Fig. 2).

Effect of E_2 or a Combination of E_2 and NMMA on LHRH Release. MBH were incubated with either NMMA, a competitive inhibitor of the enzyme NOS, or E_2 and NMMA (3×10^{-4} *M*) for 1 hr to assess the role of NO. NMMA by itself failed to alter LHRH release but a

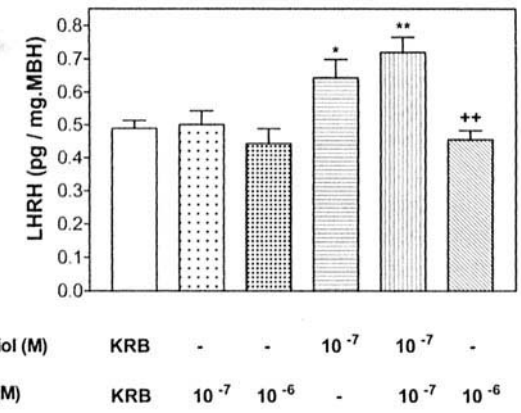

Figure 2. Influence of 17β-estradiol (E_2) or a combination of E_2 and tamoxifen on luteinizing hormone-releasing hormone (LHRH) release after 1 hr of incubation. $^{*}P < 0.05$ or $^{**}P < 0.01$ vs. Krebs-Ringer bicarbonate (KRB). ++P < 0.01 vs. the group treated with E_2. MBH, medial basal hypothalamus.

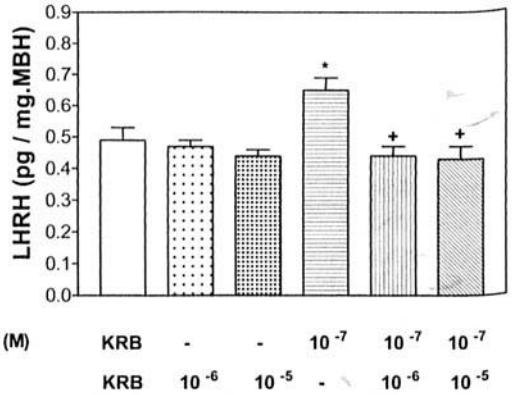

Figure 4. Influence of 17β-estradiol (E_2) or a combination of E_2 and LY 83583 on luteinizing hormone-releasing hormone (LHRH) release after 1 hr of incubation. $^{*}P < 0.05$ vs. Krebs-Ringer bicarbonate (KRB). $+P < 0.05$ vs. the group treated with E_2. MBH, medial basal hypothalamus.

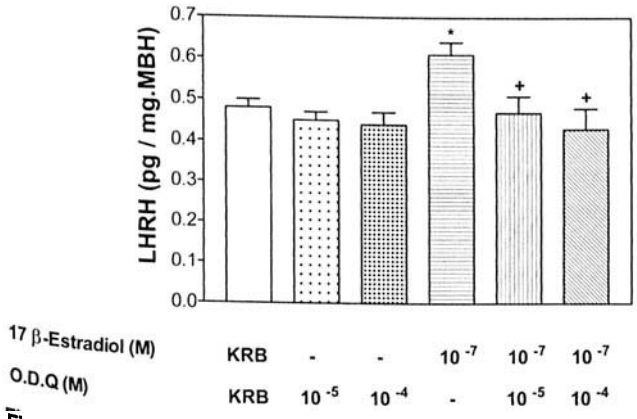

Figure 5. Effect of 17β-estradiol E_2 or a combination of (E_2) and oxadiazolo [4,3-a] quinoxalin-1-one (O.D.Q.) on luteinizing hormone-releasing hormone (LHRH) release after 1 hr of incubation. $^{**}P < 0.01$ vs. Krebs-Ringer bicarbonate (KRB). $+P < 0.05$ vs. the group treated with E_2.

combination of E_2 and NMMA significantly suppressed E_2-induced LHRH release (Fig. 3).

Influence of E_2 or a Combination of E_2 and LY 83583 on LHRH Release. MBH were incubated for 1 hr with either E_2 or a combination of E_2 and LY 83583 (10^{-6} and 10^{-5} M), an NOS inhibitor. LY 83583 at both doses (10^{-6} and 10^{-5} M) failed to alter LHRH release (Fig. 4). However, a combination of E_2 and LY 83583 significantly suppressed E_2-induced LHRH release.

Effect of E_2 or a Combination of E_2 and O.D.Q. on LHRH Release. In order to assess the role of a specific soluble GC inhibitor on E_2-induced LHRH release, the tissues were incubated with either E_2 or a combination of E_2 and O.D.Q. (10^{-5} and 10^{-4} M) for 1 hr. O.D.Q. failed to alter LHRH release and a combination of E_2 and both doses of O.D.Q. completely blocked E_2-induced LHRH release (Fig. 5).

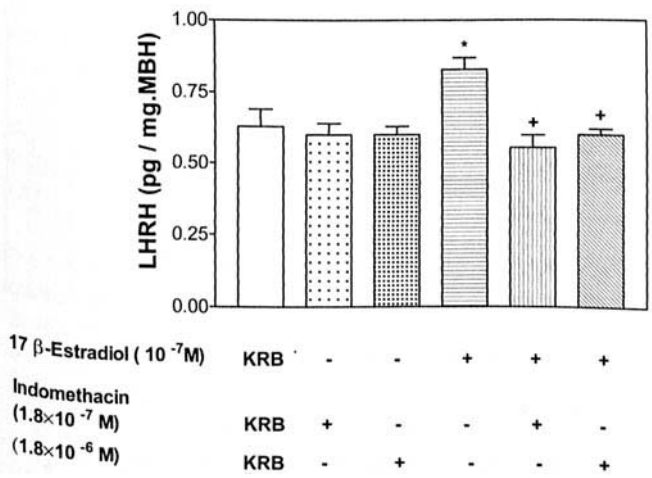

Figure 6. Effect of 17β-estradiol (E_2) or a combination of E_2 and indomethacin on luteinizing hormone-releasing hormone (LHRH) release after 1 hr of incubation. $^{*}P < 0.05$ vs. Krebs-Ringer bicarbonate (KRB). $+P < 0.05$ vs. the group treated with E_2.

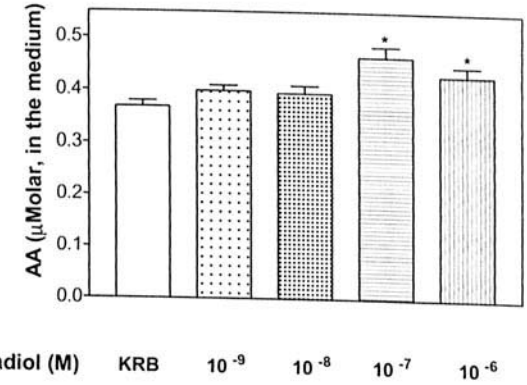

Figure 7. Effect of graded concentrations of 17β-estradiol (E_2) on ascorbic acid (AA) release after 1 hr of incubation. $^{*}P < 0.05$ vs. Krebs-Ringer bicarbonate (KRB).

Effect of E_2 or a Combination of E_2 and Indomethacin on LHRH Release. Incubation of MBH with either E_2 or a combination of E_2 and indomethacin for 1 hr showed that indomethacin (1.8×10^{-7} and 1.8×10^{-6} M) by itself failed to alter LHRH release but a combination of E_2 and indomethacin at both doses significantly suppressed LHRH release (Fig. 6).

Effect of Graded Concentrations of E_2 on AA Release. MBH were incubated with varying concentrations of E_2 (10^{-9} and 10^{-6} M) for 1 hr (Fig. 7). The lowest dose that stimulated AA release was 10^{-7} M, and a similar increase was observed at 10^{-6} M.

Influence of E_2 or a Combination of E_2 and Tamoxifen on AA Release. In order to assess the role of tamoxifen on E_2-induced AA release, the tissues were incubated with E_2 (10^{-7} M) or a combination of E_2 and tamoxifen (10^{-7} and $10^{-6}M$) for 1 hr. The results showed that tamoxifen at both doses failed to alter AA release (Fig. 8). A combination of E_2 and tamoxifen (10^{-7} M) was ineffective in lowering E_2-induced AA release, but a

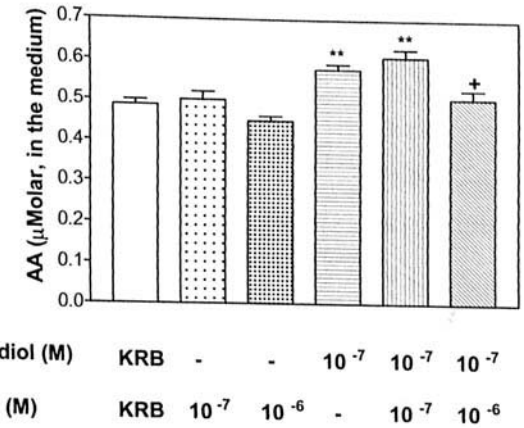

Figure 8. Influence of 17β-estradiol (E_2) or a combination of E_2 and tamoxifen on ascorbic acid (AA) release in the medium after 1 hr of incubation. $^{**}P < 0.01$ vs. Krebs-Ringer bicarbonate (KRB). $+P < 0.05$ vs. the group treated with E_2.

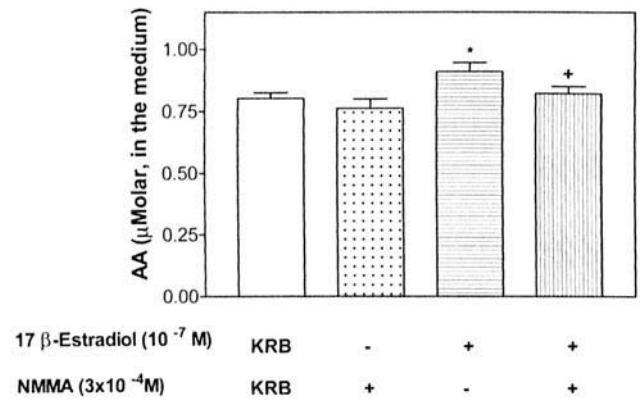

Figure 9. Effect of 17β-estradiol (E_2) or a combination of E_2 and N^G monomethyl amine (NMMA) on AA release in the medium after 1 hr of incubation. $*P < 0.05$ vs. KRB. $+P < 0.05$ vs. the group treated E_2.

combination of E_2 and a higher dose of tamoxifen (10^{-6} *M*) totally blocked E_2-induced AA release (Fig. 8).

Effect of E_2 or a Combination of E_2 and NMMA on AA Release. MBH were incubated with NMMA or E_2 plus NMMA to assess the role of NO in the release process. NMMA by itself failed to alter AA release, and a combination of E_2 plus NMMA significantly suppressed E_2-induced AA release (Fig. 9).

Effect of E_2 or a Combination of E_2 and LY 83583 on AA Release. MBH were incubated with either E_2 or a combination of E_2 and LY 83583 (10^{-6} and 10^{-5} *M*), an NOS inhibitor for 1 hr. LY 83583 at both doses significantly suppressed basal AA release and the effect was dose-dependent (Fig. 10). A combination of E_2 with either of two doses of LY 83583 significantly suppressed AA release.

Effect of E_2 or a Combination of E_2 and O.D.Q. on AA Release. In order to assess the role of soluble GC inhibitor on E_2-induced LHRH release, the tissues were

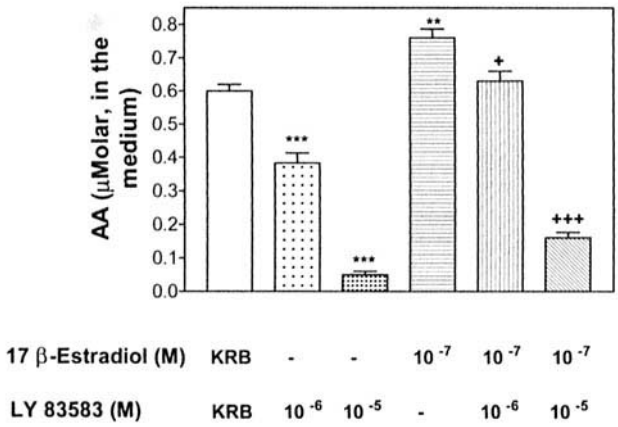

Figure 10. Effect of 17β-estradiol (E_2) or a combination of 17β-estradiol E_2 and LY 83583 on ascorbic acid (AA) release after 1 hr of incubation. $**P < 0.01$ or $***P < 0.001$ vs. Krebs-Ringer bicarbonate (KRB). $+P < 0.05$ or $+++ = P < 0.001$ vs. the group treated with E_2.

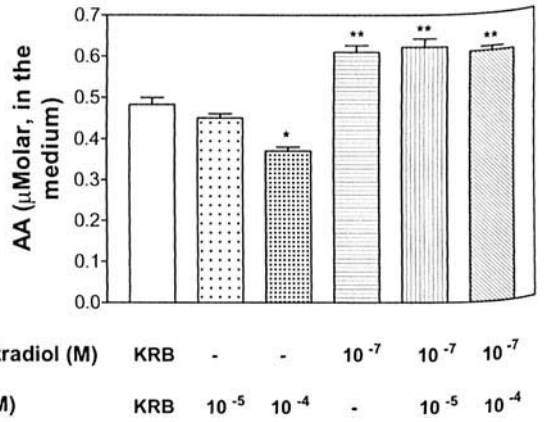

Figure 11. Effect of 17β-estradiol (E_2) or a combination of E_2 and oxadiazolo [4,3-a] quinoxalin-1-one (O.D.Q.) on ascorbic acid (AA) release after 1 hr of incubation. $*P < 0.05$ or $**P < 0.01$ vs. Krebs-Ringer bicarbonate (KRB).

to alter AA release, but the higher dose suppressed basal AA release (Fig. 11). However, a combination of E_2 and both doses of O.D.Q. failed to alter E_2-induced AA release.

Effect of E_2 or a Combination of E_2 and Indomethacin on AA Release. Incubation of MBH with either E_2 or a combination of E_2 and indomethacin for 1 hr showed that indomethacin (1.8×10^{-7} and 1.8×10^{-6} *M*) failed to alter AA release (Fig. 12). A combination of E_2 and indomethacin at both doses significantly suppressed AA release (Fig. 12).

Discussion

The present investigation demonstrates the effect of graded concentrations of E_2 on AA and LHRH release from the MBH of adult male rats. 17β-Estradiol produces a significant increase in both AA and LHRH release. The stimulatory effect was blocked in the presence of tamoxifen, an antiestrogen, suggesting that the release process is

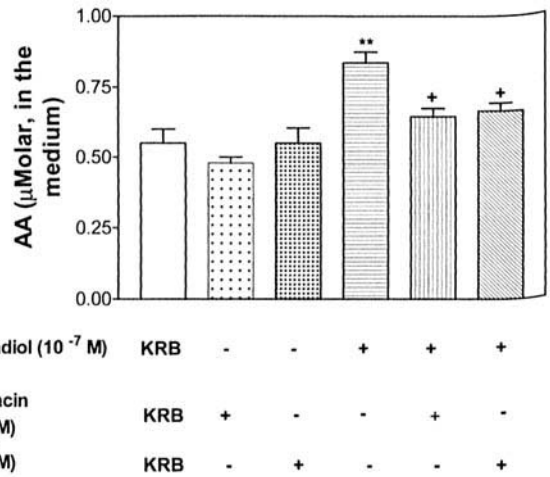

Figure 12. Effect of 17β-estradiol (E_2) or a combination of E_2 and indomethacin on ascorbic acid (AA) release after 1 hr of incubation. $**P < 0.01$ vs. Krebs-Ringer bicarbonate (KRB). $+P < 0.05$ vs. the group treated with E_2.

mediated by a specific estradiol receptor. Tamoxifen is considered to be an antagonist at the nuclear as well as the membrane receptor level (39, 40). Our data show that the inhibitory effect of tamoxifen is not observed at 10^{-7} *M*, but it is observed at a 10 times higher concentration. It is possible that a prior exposure of MBH to tamoxifen (10^{-7} *M*) followed by incubation with E_2 or a prolonged incubation with equimolar concentrations of E_2 and tamoxifen is needed for its inhibitory action. To our knowledge, this is the first report to show that E_2, an antioxidant, is able to stimulate AA, another potent antioxidant present in high concentrations in the hypothalamus (30–32). Furthermore, the ability of E_2 to stimulate AA and LHRH release is similar to that observed with other antioxidants; namely melatonin, the principal secretory product of the pineal gland, and vitamin E, a fat-soluble vitamin (41–45). E_2-stimualated AA and LHRH release is inhibited by NMMA, a competitive inhibitor of NOS, indicating that the effects are mediated by NO. Vitamin E and melatonin-induced AA and LHRH release are also suppressed by NMMA, suggesting NO mediation (41, 44). Taken together, these data suggest that LHRH stimulation via NO may be the common pathway involved in the action of some antioxidants.

Substantial evidence suggests that NO plays a crucial role in the control of LHRH secretion (46–48). Previous studies have shown that NO stimulated LHRH release from LHRH terminals in the median eminence or from immortalized LHRH neurons (46–48). The close proximity of LHRH neurons to the anatomical localization of NO neurons in the hypothalamus further supports the important role of NO in the regulation of LHRH secretion (48, 49). Several techniques have been used to investigate the effect of estrogen on LHRH secretion into the hypophyseal portal system (50–55). In sheep, the concentration of LHRH in hypothalamic portal blood is elevated at the time of LH surge that coincides with an elevation of plasma E_2 (50, 51). In female rats, E_2 has been shown to exert both stimulatory and inhibitory effects on LHRH release, depending on their plasma levels (56, 57). Incubation of MBH slices from adult ovariectomized rats with E_2 potentiated high potassium-induced LHRH release, and this was reversed by tamoxifen, suggesting that the action was mediated by the receptors (39).

The effect of E_2 on LHRH release in male rats has not been thoroughly investigated. One study using adult male quails shows that basal LHRH release is increased by an acute exposure of hypothalamic slices to E_2 (58). Incubation of median eminence fragments, including vascular tissue from adult male rats to E_2, has been shown to stimulate NO release, which in turn, stimulated LHRH release in a dose-dependent manner (59). Hemoglobin, an NO scavenger, inhibits E_2-induced NO release, indicating that the process is mediated by NO (59). Tamoxifen antagonizes the action of E_2 and abolishes E_2-stimulated LHRH release, suggesting that the stimulation is via its receptors (59), and this is consistent with our findings. In the present investigation, E_2 at 10^{-8} *M* or lower concentration is ineffective in stimulating LHRH release. However, E_2 at these concentrations has been shown to stimulate LHRH release (59). Whether or not the difference in incubation conditions is responsible for this discrepancy is not known. In this study, we incubated MBH for 1 hr because it is not possible to measure AA release after 30 mins or less. Because NO plays a major role in reproduction, it is most likely that E_2 activates NOS and releases NO, which in turn, releases LHRH. The ability of E_2 to regulate neuronal NOS in the brain and its stimulatory action on NOS has been demonstrated earlier (60, 61). Administration of estrogen to ovariectomized rats has been shown to increase neuronal NOS mRNA in the ventromedial nucleus of the hypothalamus (62). In addition, the neuronal NOS immunoreactivity in the preoptic area is influenced by estrogen, and the effect is greater in animals possessing a functional ERα gene compared with that of ERα knockout mice (60).

To further evaluate the role of NO in the action of E_2-induced LHRH and AA release we used LY 83583, a blocker of NOS that is also capable of lowering the levels of NO-dependent cGMP in tissues (34, 63, 64). In our experiment, LY 83583 significantly suppresses E_2-induced AA and LHRH release. It is also effective in lowering basal AA release, demonstrating that basal AA release involves cGMP mediation. This is comparable to our earlier studies that used LY 83583 at these concentrations (44, 65). LY 83583 has been shown to suppress neuronal NOS, and this may result in lower NO release, which in turn, lowers cGMP levels (34). We have also used O.D.Q., a selective, reversible, and competitive inhibitor of the GC targeted by NO both *in vivo* and *in vitro* (35, 66). O.D.Q. produces a differential effect on E_2-induced AA and LHRH release. It suppresses E_2-induced LHRH release, indicating that the stimulation is via soluble GC, and this is in accordance with an earlier study (59). However, O.D.Q. is ineffective in altering E_2-induced AA release. It is most likely that a prolonged incubation time or higher dose of O.D.Q is required for its inhibitory action. Because E_2-induced AA release is significantly lowered in the presence of LY 83583, and because LY 83583 has been reported to reduce tissue cGMP levels and also to inhibit soluble GC, we infer that cGMP plays a role in E_2-induced AA release (63, 67).

We have shown previously the ability of AA to suppress *N*-methyl D-aspartic acid (NMDA)-induced LHRH release without modifying the resting release (33). In the present investigation, AA had no effect on E_2-induced LHRH release. Recently, a similar and parallel increase in AA and LHRH release with vitamin E and melatonin has been reported (41, 44). It is not clear why the action of AA in the presence of NMDA is different from its action in the presence of antioxidants. Further work is needed to understand the precise and differential action of AA. Collectively, our results suggest that cGMP is involved in E_2-induced LHRH release. O.D.Q., a selective inhibitor of

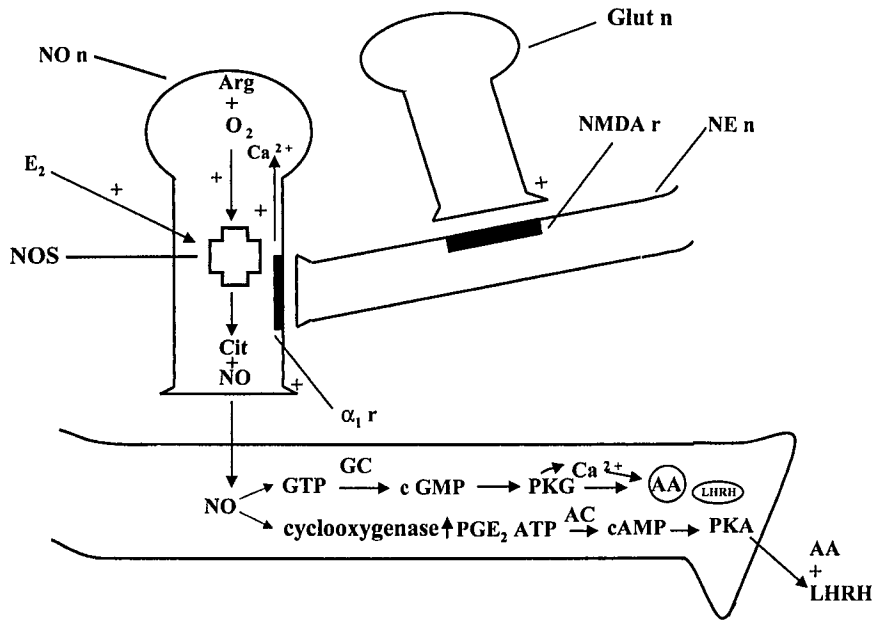

Figure 13. A schematic diagram of the hypothesized mechanism of action of 17β-estradiol (E_2) on ascorbic acid (AA) and luteinizing hormone-releasing hormone (LHRH) release. Glut n, glutamergic neuron; NMDA r, NMDA receptor; NE n, norepinephrine neuron; NO n, NOergic neuron; Arg, arginine; O_2, oxygen; cit, citrulline; NO, nitric oxide; α_1 r, α_1 receptor; GTP, guanosine triphosphate; or +, stimulation; GC, guanylyl cyclase; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; Ca^{2+}, calcium; PGE_2, prostaglandin E_2; ATP, adenosine triphosphate; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A.

the soluble GC and LY 83583, an inhibitor of NOS, produce similar results as far as E_2-induced LHRH release is concerned. This suggests that the final effect with both the inhibitors is suppression of cGMP formation, although their targets are at different stages of the NO/cGMP signaling pathway.

Incubation of MBH with a combination of E_2 and indomethacin, a prostaglandin synthesis blocker, results in suppression of both AA and LHRH release, suggesting that prostaglandins are involved as intracellular mediators of E_2-induced AA and LHRH release. Our results are summarized in Figure 13. We hypothesize that the axons of glutamatergic neurons synapse on the axons of the noradrenergic neurons, which in turn, synapse on the NOergic neurons. NMDA causes the release of norepinephrine. This combines with α_1-noradenergic receptors and increases intracellular calcium and stimulates NOS and releases NO. NO, in turn, activates the soluble GC and increases cGMP that will increase the activity of protein kinase G. Protein kinase G will increase intracellular calcium and induce the exocytosis of AA and LHRH. NO will also increase the activity of cyclooxygenase that will catalyze the conversion of arachidonic acid to prostaglandin E_2. Prostaglandin E_2, in turn, will increase cAMP and activate protein kinase A and finally induce the exocytosis of LHRH secretory granules (68–70). We conclude that E_2 stimulates both AA and LHRH release, and that the release is mediated by NO, cGMP, and prostaglandin. 17β-Estradiol plays a role in the hypothalamic control of AA and LHRH release and its antioxidant effect may be mediated via AA.

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