PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 174, 284–290 (1983)

Catecholamine Mechanisms in the Feedback Effects of Estradiol Benzoate on the Release of LH and Prolactin[1] (41738)

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Abstract. The role of hypothalamic catecholamines and luteinizing hormone releasing hormone (LHRH) in the negative feedback effect of estradiol benzoate (EB) on luteinizing hormone (LH) release was studied in chronic ovariectomized rats. Administration of 10 μg EB decreased plasma LH levels and increased LHRH content in the medial basal hypothalamus (MBH) 1 day after injection. Inhibition of dopamine and norepinephrine synthesis with α-methyl-*p*-tyrosine (α-MT) reduced the LHRH content in the MBH in both oil- and EB-treated animals and partially reversed the decrease in plasma LH levels. Inhibition of norepinephrine synthesis with fusaric acid decreased LHRH content in both oil- and EB-treated rats but had no effect on plasma LH levels. The results suggest that at least a portion of the inhibitory effect of EB on LH release is due to the stimulation of an inhibitory dopaminergic mechanism which reduces LHRH release from the MBH. This feedback mechanism is apparently not susceptible to dopaminergic receptor blockade since administration of pimozide had no effect on LH levels. The stimulatory feedback effect of EB on prolactin release was studied in the same animals. α-MT and EB produced additive effects on plasma prolactin levels whereas fusaric acid blocked the EB-induced increase in plasma prolactin levels. Pimozide appeared to potentiate the effect of EB on prolactin release. The results reconfirm the possible role of noradrenergic neurons in the release of prolactin induced by EB and also suggest that EB stimulates a dopaminergic mechanism which is inhibitory to prolactin release but is normally masked by increased noradrenergic activity.

Estradiol is known to produce positive and negative feedback effects on luteinizing hormone (LH) release in the ovariectomized rat (1). There is considerable evidence that dopaminergic and/or noradrenergic pathways terminating in the hypothalamus may be involved in these feedback actions (2). With regard to inhibitory feedback, administration of estrogen has been reported to decrease norepinephrine turnover in various hypothalamic nuclei and decrease plasma LH levels concurrently (3, 4), whereas tyrosine hydroxylase activity (5) and dopamine turnover (4) are increased.

The actions of hypothalamic catecholamines on LH release are probably mediated through hypothalamic luteinizing hormone releasing hormone (LHRH). It has been proposed that noradrenergic neurons synapse with LHRH-containing neurons in the preoptic area (6, 7) and that dopaminergic neurons terminating in the external layer of the median eminence make axoaxonic contact with LHRH neurons (8). Addition of norepinephrine to *in vitro* preparations of medial basal hypothalamus (MBH) has been reported to have no effect (9) or to stimulate (10) LHRH release. Inhibition of norepinephrine synthesis by diethyldithiocarbamate led to a decrease in LHRH levels in the MBH (11). *In vitro* studies have shown that dopamine can induce release of LHRH from the MBH (9, 12, 13). Although the dopamine receptor antagonist pimozide has been reported to have no effect on release of LHRH *in vitro*, it has been shown to block dopamine-stimulated LHRH release (9, 10). The contradictory results of such *in vitro* studies may be due to neuronal degeneration in fragments of hypothalamic tissues (14).

Despite the aforementioned studies, it has not been completely resolved whether the negative feedback action of estrogen in the hypothalamus which leads to inhibition of LH release occurs via an effect on catecholamine neurons and subsequent alteration of LHRH release or by some other mechanism (14). Although estradiol has been shown to reverse the fall in LHRH content in the MBH fol-

[1] Supported by Public Health Service Grant HD 11922.

0037-9727/83 $1.50

lowing ovariectomy (15) it is not clear whether this effect is directly related to alterations in catecholamine neuron activity. It is apparent that more physiological studies are needed to answer these questions (14).

Estrogen is known to stimulate prolactin release in the ovariectomized rat (7, 16), possibly via stimulation of hypothalamic noradrenergic neuron activity (17). This may be open to question, however, since estrogen can also enhance dopamine turnover in the MBH (4, 7).

In the present study, the effects of a number of drugs which alter catecholamine neuron function were studied to further clarify the role of catecholamines and LHRH in the inhibitory feedback action of estradiol on LH release in the ovariectomized rat. Additional studies were carried out to elucidate the role of catecholamines in estrogen-stimulated prolactin release.

Materials and Methods. *Animals.* Mature female Sprague–Dawley rats weighing 180–200 g (Laboratory Supply Co., Indianapolis, Ind.) were housed five to a cage in a temperature-controlled room (25 ± 2°C) with lights on from 5 AM to 7 PM daily. Water and Purina Rat Chow were supplied *ad libitum.* The animals were ovariectomized under ether anesthesia and used 3–4 weeks after surgery.

Experimental procedure. Ovariectomized animals were injected subcutaneously with either 10 μg estradiol benzoate (EB; Sigma) in 0.1 ml peanut oil or 0.1 ml peanut oil alone at 9 AM. The following drugs or their vehicles were administered the next day: fusaric acid (Sigma) in saline, 100 mg/kg sc at 11 AM; pimozide (McNeil) in tartaric acid or ethanol, 1 mg/kg sc or α-methyl-*p*-tyrosine methyl ester HCl (α-MT; Sigma) in saline, 250 mg/kg ip at 12 noon. Previous studies in our laboratory (18) have shown that this dose of fusaric acid produces a significant inhibition of dopamine-β-hydroxylase in the MBH. Additional studies (unpublished) have indicated that this dose of α-MT produces a significant decrease in tyrosine hydroxylase activity in the MBH when measured *in vitro* 2 hr after drug administration.

Groups of rats were killed by decapitation at 2 PM, 2 or 3 hr after drug administration. Trunk blood was collected for radioimmunoassay of LH and prolactin. The brain was removed and medial basal hypothalamus (10–15 mg) was dissected for assay of LHRH. The tissue was added to tubes containing 1 ml of Krebs–Ringer bicarbonate buffer (19), and 10 μl 6×10^{-2} *M* ascorbic acid and 10 μl 1×10^{-3} *M* bacitracin to prevent peptidasic degradation of LHRH (12). The samples were sonicated with a Kontes cell disruptor for 20 sec and aliquots frozen until assayed.

LH and prolactin assay. Trunk blood was obtained following decapitation and the plasma separated from the cells and stored at −20°C for subsequent assay. Each plasma sample was assayed in duplicate or triplicate for prolactin and LH by the radioimmunoassay methods of Niswender *et al.* (20). The antiovine LH serum for the ovine LH for iodination (LER-1056 C2) was kindly provided by Dr. Leo Reichert. The remainder of the assay materials was provided by the NIAMDD Hormone Distribution Program. The reference preparations used were NIAMDD rat prolactin-RP-1 and NIAMDD rat LH-RP-1.

LHRH assay. Anti-LHRH antisera (r-42) was supplied by Dr. Gordon Niswender. LHRH for iodination and LHRH reference preparation were obtained from Abbott Laboratories, North Chicago, Illinois.

Iodination of the peptide was done as described above for LH and prolactin. The $Na^{125}I$, mono-iodo-LHRH, and di-iodo-LHRH were separated on a column of QAE Sephadex (Q-25-120) using 0.1 *N* imidazole buffer (pH 9.2) for elution (20).

Antisera, labeled LHRH and unknowns or standards were mixed and incubated at 24 hr at 4°C, after which 1 ml cold ethanol was added. This mixture was vortexed and centrifuged at 2000*g* for 30 min. The supernate containing the free labeled LHRH was aspirated and the precipitate containing the bound labeled LHRH was counted in an automatic γ counter. Calculations were performed as described above for LH.

Data analysis. All hormone data were analyzed by one-way analysis of variance and by Student's *t* test.

Results. *Effects of EB and αMT on LHRH, LH, and prolactin.* Treatment with EB alone significantly elevated the content of LHRH in the MBH 29 hr after injection compared to vehicle controls (Table I). Plasma LH levels were dramatically decreased by EB treatment

TABLE I. EFFECTS OF α-METHYL-*p*-TYROSINE AND ESTRADIOL BENZOATE ON LHRH CONTENT OF THE MBH AND ON PLASMA LH AND PROLACTIN

Treatment	LHRH (pg/MBH)	LH (ng/ml)	Prolactin (ng/ml)
oil + saline	634 ± 65 (14)[a]	831 ± 72 (8)	10 ± 1 (11)
EB + saline	950 ± 96[b] (12)	204 ± 35[b] (13)	52 ± 8[b] (14)
oil + αMT	334 ± 82[b] (7)	928 ± 99 (15)	98 ± 14[b] (11)
EB + αMT	383 ± 59[c] (7)	469 ± 68[c] (19)	183 ± 21[c] (15)

Note. Rats were administered 10 μg EB or oil at 9 AM on Day 1 and αMT (250 mg/kg) or saline at 12 noon on Day 2. Tissue LHRH content and plasma LH and prolactin levels were determined in samples obtained at 2 PM on Day 2.

[a] Values represent means ± SEM; number of animals in parentheses.

[b] Significantly different from oil + saline ($P < 0.05$).

[c] Significantly different from EB + saline ($P < 0.05$).

during the same time interval, while plasma prolactin levels were significantly increased.

To determine whether brain catecholamines are involved in maintaining the basal levels of LHRH, LH, or prolactin or are involved in the alterations caused by EB, additional groups were given α-MT. As noted in Table I, the content of LHRH in the MBH was significantly reduced by administration of α-MT in both the steroid-primed and oil-treated groups. Although α-MT partially blocked the decrease in plasma LH levels caused by EB, it had no effect on LH in oil-treated animals. Treatment with α-MT alone caused a marked increase in plasma prolactin levels which was further enhanced by priming with EB, suggesting that α-MT and EB have additive effects on prolactin release.

Effects of EB and fusaric acid on LHRH, LH, and prolactin. To determine whether norepinephrine or dopamine specifically is involved in the feedback actions of estradiol on the release of LHRH, LH, and prolactin, the dopamine-β-hydroxylase inhibitor, fusaric acid, was administered to rats pretreated with oil or EB. Treatment with fusaric acid alone decreased the content of LHRH in the MBH (Table II). LHRH content was also significantly reduced by fusaric acid in the EB-treated rats. Despite these effects on LHRH levels, plasma LH levels were not altered by fusaric acid. Although fusaric acid by itself had no effect on plasma prolactin levels, prolactin levels were dramatically decreased when FA was administered to rats pretreated with EB.

Effects of pimozide and EB on LH and prolactin. The actions of pimozide, a dopaminergic receptor blocking agent, were studied to further elucidate the role of dopaminergic

TABLE II. EFFECTS OF FUSARIC ACID AND ESTRADIOL BENZOATE ON LHRH CONTENT OF MBH AND ON PLASMA LH AND PROLACTIN

Treatment	LHRH (pg/MBH)	LH (ng/ml)	Prolactin (ng/ml)
oil + saline	634 ± 65 (14)[a]	831 ± 72 (8)	10 ± 1 (11)
EB + saline	950 ± 96[b] (12)	204 ± 35[b] (13)	52 ± 8[b] (14)
oil + FA	370 ± 51[b] (8)	850 ± 94 (13)	8 ± 2 (12)
EB + FA	608 ± 122[c] (8)	279 ± 34 (12)	14 ± 4[c] (12)

Note. Rats were administered 10 μg EB or oil at 9 AM on Day 1 and FA (100 mg/kg) or saline at 11 AM on Day 2. Tissue LHRH content and plasma LH and prolactin levels were determined in samples obtained at 2 PM on Day 2.

[a] Values represent means ± SEM; number of animals in parentheses.

[b] Significantly different from oil + saline ($P < 0.05$).

[c] Significantly different from EB + saline ($P < 0.05$).

mechanisms in the feedback actions of EB on LH and prolactin release following treatment with EB.

Ethanol was initially utilized as a vehicle for pimozide. Although ethanol had no effect on plasma prolactin levels, it did increase plasma LH levels when compared with EB-treated controls (Table III). Pimozide appeared to slightly enhance this effect and to further increase plasma prolactin levels. To avoid these confounding actions of ethanol, tartaric acid was subsequently used as a vehicle for pimozide since it had no effect on LH or prolactin levels in EB-treated animals (Table III). Administration of pimozide in tartaric acid had no discernible effect on plasma LH levels. However, prolactin levels were again significantly elevated by pimozide.

Since ethanol was found to elevate plasma LH levels significantly in EB-treated animals, a further study was carried out to determine whether this effect was due to an action on LHRH. The content of LHRH in the MBH was significantly reduced in the EB + ethanol group compared to controls (Table IV). This change in LHRH content was accompanied by significant increases in plasma LH to the control levels observed in oil-treated rats.

Discussion. Administration of EB to ovariectomized rats in the present study caused a significant increase in the concentration of LHRH in the MBH, in agreement with others (15, 22). Several mechanisms, including inhibition of release, increased synthesis and decreased degradation of LHRH, could have accounted for this effect. Hypothalamic arylamidase has been proposed to be involved in the feedback effects of LH and steroids on LHRH release (23). However, it is unlikely that a decrease in the activity of hypothalamic arylamidase was a major factor since estradiol has been shown to stimulate arylamidase activity in ovariectomized rats (23). Although an increase in LHRH synthesis is possible, Moguilevsky *et al.* (24) reported that hypothalamic LHRH synthesis was greater in castrated animals than in intact rats, suggesting that estrogen may inhibit LHRH synthesis. Thus, interference with LHRH release appears to be the most likely mechanism to account for the increase in LHRH levels in the MBH and the subsequent decrease in plasma LH levels.

A number of studies suggest that hypothalamic catecholamine neuronal systems are involved in the inhibitory effects of EB on both LHRH and LH release. It has recently been reported that EB causes an increase in tyrosine hydroxylase activity in the MBH 1 day after treatment when plasma LH is markedly decreased (5) and that EB can increase dopamine turnover in the median eminence (25, 26) and decrease norepinephrine turnover in the suprachiasmatic preoptic region (27). Since most recent studies suggest that dopamine inhibits LH release (28) and dopaminergic neurons appear to synapse on LHRH neurons, it seems likely that EB may inhibit LHRH release via

TABLE III. EFFECT OF PIMOZIDE AND VEHICLES ON EB-INDUCED CHANGES IN PLASMA LH AND PROLACTIN

Treatment	LH (ng/ml)	Prolactin (ng/ml)
EB + saline	204 ± 35 (13)[a]	52 ± 8 (14)
EB + ethanol	634 ± 112[b] (11)	44 ± 8 (13)
EB + pimozide (ethanol)	943 ± 187[b] (15)	162 ± 35[c] (15)
EB + tartaric acid	293 ± 66 (5)	43 ± 8 (4)
EB + pimozide (tartaric acid)	273 ± 39 (6)	124 ± 3[d] (5)

Note. Rats were administered 10 μg EB or oil 9 AM on Day 1 and either pimozide, 1 mg/kg, in ethanol or tartaric acid or the vehicles at 11 AM on Day 2. Plasma hormone levels were determined in samples obtained at 2 PM on Day 2.

[a] Values represent mean ± SEM; number of animals in parentheses.

[b] Significantly different from EB + saline ($P < 0.05$).

[c] Significantly different from EB + ethanol ($P < 0.05$).

[d] Significantly different from EB + tartaric acid ($P < 0.05$).

TABLE IV. EFFECT OF ETHANOL ON LHRH CONTENT OF THE MBH AND PLASMA LH IN EB-TREATED RATS

Treatment	LHRH (pg/MBH)	LH (ng/ml)
EB + saline	950 ± 96 (12)[a]	204 ± 35 (13)
EB + EtOH	342 ± 68[b] (7)	634 ± 112[b] (11)

Note. Rats were administered 10 μg EB or oil at 9 AM on Day 1 and ethanol (1 ml/kg) or saline at 11 AM on Day 2. Tissue content of LHRH and plasma levels of LH were determined in samples obtained at 2 PM on Day 2.

[a] Values represent means ± SEM; numbers of animals in parentheses.

[b] Significantly different from EB + saline ($P < 0.05$).

stimulation of dopaminergic neurons and subsequent hyperpolarization of the LHRH neuron (29).

The results of the present study provide further evidence that the inhibitory effects of EB on LHRH and LH release may be mediated by an inhibitory dopaminergic pathway acting on LHRH neurons. For example, administration of α-MT to both vehicle and EB-treated rats caused a significant reduction in tissue content of LHRH when compared to their respective controls. It is unlikely that α-MT affected LHRH degradation directly since enhanced degradation requires the presence of gonadal steroids (30). On the other hand, it is possible that treatment with α-MT caused an increase in LHRH release by preventing the inhibitory action of the dopaminergic neurons. The fact that α-MT also partially reversed the action of EB on LH release further suggests that α-MT altered LHRH release and not synthesis nor metabolism.

The effects caused by inhibition of norepinephrine synthesis with fusaric acid also suggest that the inhibitory action of EB on LH release is due to a dopaminergic rather than a noradrenergic mechanism, since this synthesis inhibitor had no effect on LH levels either by itself or in EB-treated rats. The effects of fusaric acid on LHRH, however, appear to contradict this hypothesis since LHRH levels in the MBH were somewhat reduced in both vehicle- and EB-treated rats. One possible explanation for this finding is that an increase in plasma LH results only when a large decrease in LHRH content occurs, e.g., EB vs EB + α-MT. This suggests that a large amount of LHRH release (greater than 200–300 pg/MBH) is required before reaching a threshold for inducing LH release following EB treatment. Such a mechanism could also explain why plasma LH levels were not increased by α-MT or fusaric acid in oil-treated animals.

If an inhibitory dopaminergic mechanism were involved in the inhibition of LH release following treatment with EB, then administration of a dopamine receptor antagonist such as pimozide would be expected to reverse this effect. However, pimozide did not alter LH levels in rats pretreated with EB. This is in agreement with other studies which showed that dopamine receptor blockade does not increase plasma LH levels in ovariectomized rats (31, 32). Thus, although dopamine appears to play a role in the negative feedback effects of EB on LH release, this mechanism apparently does not involve dopamine receptors. This is supported by the failure to demonstrate dopamine agonist or antagonist binding sites in the basal hypothalamus (33) and the fact that tuberoinfundibular dopamine neurons have no autorecptors which are characteristic of other dopaminergic neuron systems (34).

Ethanol has been reported to produce biphasic dose-dependent effects on LH release in male rats (35). Low doses (0.5–1.0 g/kg), similar to that administered in the present study, increased serum LH levels whereas doses of 1.5 g/kg or higher decreased LH levels. These effects are considered to result from a direct action of ethanol in the hypothalamus since pituitary sensitivity to LHRH remains unchanged (35). The present study offers more direct evidence for a hypothalamic site of action since a marked decrease in the LHRH content of the MBH was observed 2 hr after administration of ethanol to EB-treated rats. Haloperidol was reported to have no effect on the suppression of plasma LH levels by ethanol (31), suggesting that this effect of ethanol is not related to changes in dopaminergic activity. The results in the present study extend this hypothesis further in that dopaminergic blockade by pimozide did not alter the ethanol-induced rise in plasma LH seen in EB-treated ovariectomized female rats.

Administration of α-MT to EB-treated rats appeared to potentiate the elevation of plasma prolactin levels. A previous study (17) indicated that the increase in prolactin release induced by EB is due in part to an increase in hypothalamic noradrenergic neuron activity. This was confirmed in the present study since inhibition of norepinephrine synthesis by fusaric acid completely blocked the effect of EB. Therefore, it appears likely that the potentiating effect of α-MT on prolactin levels was caused primarily by inhibition of dopamine synthesis which was previously elevated by EB (16). Combined treatment with pimozide and EB also led to a greater level of plasma prolactin (124 ng/ml), compared to either pimozide (52 ng/ml) or EB (52 ng/ml) alone, further supporting the hypothesis that dopaminergic inhibitory influence on prolactin release is elevated following treatment with EB.

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Received February 9, 1983. P.S.E.B.M. 1983, Vol. 174.