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Persistence of Low Hypothalamic Dopaminergic Activity after Removal of Chronic Estrogen Treatment (42227)

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Abstract. The purpose of this study was to determine whether inhibition of tuberoinfundibular dopaminergic (TIDA) neuron function which occurs during chronic estrogen administration persists after removal of the estrogen. Ovariectomized (OVX) Fischer 344 (F344) rats were implanted for 4 weeks with a Silastic capsule containing estradiol-17β (E_2) and controls with an empty capsule for 4 weeks. Other rats which received E_2 for 4 weeks had the capsule removed and experiments performed 4 weeks later. At the end of 4 weeks of E_2 treatment, anterior pituitary (AP) weight was increased sixfold, serum prolactin (PRL) 65-fold, and AP DNA content fivefold over OVX control rats. Four weeks after removal of E_2, AP weight, serum PRL, and AP DNA content declined, but remained significantly above OVX control values. At the end of 4 weeks of E_2 treatment and after E_2 withdrawal, release of [^{3}H]dopamine (DA) from median eminence (ME) tissue superfused *in vitro* was lower than from ME of OVX control rats although [^{3}H]DA accumulation was not significantly different among the treatment groups. Administration of apomorphine (APO), a dopamine agonist, significantly reduced plasma prolactin levels in OVX control rats, in rats at the end of 4 weeks E_2 treatment, and in rats after 4 weeks of E_2 withdrawal. Injection of haloperidol (HALO) produced similar increases in plasma PRL/estimated PRL-cell DNA in OVX controls, at the end of E_2 treatment or after E_2 withdrawal. However, injection of morphine (MOR), a drug which increases the release of PRL by inhibiting hypothalamic dopaminergic activity, resulted in a rise in plasma PRL/estimated PRL-cell DNA in OVX control rats that was significantly greater compared to rats at the end of E_2 treatment or after E_2 withdrawal. Since rats treated with E_2 released less [^{3}H]DA from ME tissue *in vitro*, and were less responsive to MOR, it can be that animals treated for 4 weeks with E_2 show a decreased ability to release DA from TIDA neurons which persists even after termination of E_2 treatment. These results suggest that chronic high circulating E_2 levels result in a depression of TIDA neuronal activity which is sustained after E_2 is removed. © 1986 Society for Experimental Biology and Medicine.

Dopaminergic neurons originating in the periventricular and arcuate nuclei of the hypothalamus send terminals to the external layer of the median eminence (ME) and constitute the tuberoinfundibular dopaminergic (TIDA) neuronal system (1, 2). These neurons secrete dopamine (DA) into the hypophysial portal vasculature and inhibit the synthesis and release of prolactin (PRL) from the anterior pituitary (AP). Short-term hyperprolactinemia increases TIDA neuron activity, resulting in short-loop feedback inhibition of PRL secretion (3, 4). However, during chronic hyperprolactinemia, dopaminergic activity may be depressed and the effects of DA on AP PRL release may be reduced (5–7).

Long-term estrogen administration in rats produces chronic hyperprolactinemia and development of PRL-secreting adenomas (8–10). However, the effects of chronic estrogen treatment on hypothalamic dopaminergic control of PRL release still remain unclear. There is evidence that long-term estrogen treatment can result in (a) cytopathological changes in the arcuate nucleus, including an increase in number of reactive glial cells and the appearance of axonal and dendritic degeneration (11, 12), (b) reduced neuronal catecholamine fluorescence in the ME and arcuate nucleus (5), (c) depletion of DA content from the ME and reduction of DA concentration in hypophysial portal blood (13, 5, 14), and (d) decreased turnover of DA and an attenuated ability to release [^{3}H]DA from ME tissue *in vitro* (14, 15).

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The decline in TIDA activity during chronic estrogen treatment may result from loss of dopaminergic neurons of the arcuate nucleus (5) or loss in sensitivity of TIDA neurons to the increased levels of circulating PRL (15). The former suggests that a permanent decline in TIDA function may occur, and persist even after removal of the estrogen treatment, whereas the latter suggests the possibility that TIDA function may be restored after estrogen removal.

To clarify this problem, the present study was undertaken to assess TIDA neuronal activity at the end of 4 weeks of estrogen treatment, and 4 weeks after removal of chronic estrogen treatment. TIDA neuronal activity was evaluated by two methods (a) *in vitro* superfusion and electrical stimulation of ME tissue after allowing for accumulation of [^{3}H]DA, and (b) testing the effectiveness of drugs that inhibit or stimulate PRL release through dopaminergic or antidopaminergic mechanisms.

Materials and Methods. *Animals and E_2 treatment.* Three-month-old female Fischer 344 (F344) rats were purchased from Harlan Industries (Indianapolis, Ind.). They weighed 160–180 g each and were housed under light and temperature controlled conditions (lights on 0500–1900 hr, 26 ± 2°C), with free access to rat chow (Ralston Purina, St. Louis, Mo.) and tap water. The animals were ovariectomized (OVX) prior to treatment and randomly divided into three groups: (a) OVX controls received (sc) an empty Silastic capsule (Dow Corning, Midland; Mich.; 10 mm in length, 0.078″ i.d. × 0.125″ o.d.) for 4 weeks after which the capsule was removed for 4 weeks, (b) rats OVX for 4 weeks and then implanted with a Silastic capsule containing 5–6 mg estradiol-17β (Sigma Chemical Co., St. Louis, Mo.; E_2) for 4 weeks, and (c) OVX rats implanted with an E_2 capsule for 4 weeks, followed by removal of the E_2 capsule for 4 weeks.

Evaluation of TIDA activity in vitro. TIDA activity was estimated by determining the release of ^{3}H from ME tissue after allowing for accumulation of [^{3}H]DA into the tissue as previously described (16). All experiments were performed beginning at 1100 hr. The rats were decapitated, trunk blood was collected for radioimmunoassay (RIA) of luteinizing hormone (LH) and PRL, and ME tissue was dissected and placed in ice cold Krebs-Henseleit medium. This tissue was preincubated for 5 min (37°C, pH 7.4, under constant 95% O_2, 5% CO_2) and then incubated for 20 min in medium containing 0.36 μM [^{3}H]DA (Amersham, Chicago, Ill.). 0.1 μM desipramine and 12.5 μM nialamide (Sigma Chemical Co.). The tissues were washed, two MEs were placed into each of two chambers, and superfusion was carried out with medium containing 12.5 μM nialamide. Fractions were collected (0.6 ml/2 min), and release of ^{3}H was expressed as a fractional rate constant. The fractional rate constant was calculated by dividing the radioactivity released (cpm) by the radioactivity in the tissue at the start of each 2-min period (fractional rate constant = 10^{-3}/min). After a 40-min washout period, the tissue was electrically stimulated using field stimulation (biphasic square wave pulse for 15 sec, 6 mA, magnitude, 2-msec duration, at 20 Hz). The stimulation-evoked release of ^{3}H was calculated by subtracting the two fractional rate constants before electrical stimulation (baseline area) from the two fractional rate constants after stimulation (peak area). Total accumulation was expressed as counts per minute per gram ME protein as measured by the Bio-Rad assay (17).

PRL response to drugs which affect dopaminergic control of PRL release. The capacity of pharmacological agents which act either on TIDA neurons or on dopaminergic receptors on the AP to stimulate or inhibit PRL release was assessed. The following three drugs were injected: apomorphine hydrochloride (APO: Sigma Chemical Co.), a DA receptor agonist; haloperidol (HALO, McNeil Laboratories, Ft. Washington, Pa.), a DA receptor antagonist; and morphine sulfate (MOR, Mallenkrodt Labs, St. Louis, MO), which inhibits central TIDA turnover (18, 19).

Different animals were used for each of three experiments. The rats were implanted with an intraatrial Silastic cannula under ether anesthesia as previously described (20). Two days later, the animals were adapted to the experimental room for 2 hr starting at 0800 hr. Blood samples were withdrawn 40 and 20 min prior to drug administration. After sc injection of APO (0.25 mg/kg, in saline) or iv injection of MOR (5 mg/kg, in saline), samples were withdrawn 15, 30, 60, and 90 min later. After sc administration of HALO (0.5 mg/kg,

in 0.3% tartaric acid), blood samples were withdrawn 45, 90, 135, and 180 min later. Dosages were chosen which produce maximal effects on PRL release in control male rats (21). Blood samples (0.6 ml) were centrifuged, plasma was separated and stored frozen at −20°C, and the erythrocytes were resuspended in saline and immediately reinjected into the animal from which the blood was removed.

Assays. Serum and plasma PRL, LH, and growth hormone (GH) were measured by radioimmunoassay (RIA) with reagents provided by the National Pituitary Agency of the NIADDK, with the exception that anti-rat PRL was provided by Dr. C. L. Chen (University of Florida). IgGSorb (Enzyme Center, Boston, Mass.) was used to separate bound from free hormone. All samples were assayed for PRL in quadruplicate using duplicates at two different dilutions. Plasma and serum hormone concentrations were expressed in terms of NIADDK rPRL-RP-3, rLH-RP-1 and rGH-RP-1. Intraassay and interassay coefficients of variation were 6.8 and 10.8% for PRL, 4.5 and 7.5% for LH, and 5 and 9% for GH, respectively. The minimum detectable doses for the PRL, LH and GH RIAs were 0.09, 0.33, and 0.05 ng/tube, respectively. The DNA assay was performed by the method of Burton (22).

Statistical analysis. Absolute values of the *in vivo* data were analyzed by one-way ANOVA followed by the Student–Newman–Keuls' multiple comparison test. Since basal serum PRL levels among groups were different, data were also expressed as ng PRL/ml plasma/estimated DNA of PRL-cells (μg) ratio. The Wilcoxson–Mann–Whitney analysis tested differences among groups when data were expressed as a ratio. For the RIA data of Table II, both the PRL and LH results revealed a significant F_{max} test for heterogeneity of variance. Therefore, data were transformed logarithmically before performing the ANOVA and Student–Newman–Keuls multiple comparison test. The *in vitro* ME superfusion results were analyzed by the Wilcoxson–Mann–Whitney test (23).

Results. Table I shows that at the end of 4 weeks of E_2 treatment (Group 2) and after 4 weeks of withdrawal from E_2 (Group 3) there was a suppression of stimulation-evoked release of [^{3}H]DA from the ME, as compared to values in OVX controls ($P \leq 0.05$). There was a trend for reduced [^{3}H]DA accumulation after 4 weeks of E_2 and after the 4-week withdrawal period which was not statistically significant ($P \geq 0.05$).

TABLE I. DECLINE IN TIDA NEURON ACTIVITY AT END OF CHRONIC E_2 TREATMENT AND AFTER WITHDRAWAL OF E_2

	Stimulation-evoked release ($\times 10^{-3}$/min)	[^{3}H]DA accumulation (cpm/μg ME protein)
1. OVX controls[a]	5.4 ± 1.5	1723 ± 230
2. End of E_2 treatment	1.8 ± 0.8*	1423 ± 100
3. After E_2 withdrawal	2.8 ± 0.6*	1406 ± 228

[a] X ± SEM of six experiments (two MEs/experiment); Group 1 was OVX for 8 weeks, Group 2 received E_2 for 4 weeks, Group 3 received E_2 for 4 weeks after which E_2 was withdrawn for 4 weeks.
* $P \leq 0.05$ compared to Group 1.

The absolute values of plasma PRL following administration of APO, HALO, and MOR to the three treatment groups are shown in Table II. APO, a DA receptor agonist, produced a significant decline in plasma PRL values 30 and 90 min after injection in OVX controls, and a significant reduction in plasma PRL levels at all time points after injection into rats treated with E_2 for 4 weeks and in rats after E_2 removal for 4 weeks ($P \leq 0.05$). Injection of HALO, a DA receptor antagonist, produced an increase in circulating levels of PRL in all groups ($P \leq 0.05$). After administration of MOR, a drug which decreases TIDA activity, plasma PRL was increased at 15 and 30 min but returned to baseline levels by 60 min in OVX control rats. Plasma PRL increased only 30 min after MOR injection in animals at the end of 4 weeks of E_2 treatment. There was a significant increase in PRL at all time points after MOR administration in rats after E_2 removal for 4 weeks.

The PRL response to pharmacological agents is also presented as the difference between the preinjection and postinjection level of PRL divided by an estimate of the portion of total AP DNA content which constitutes only the PRL-secreting cells (Fig. 1). Assuming blood volume and clearance of the hormone are similar among treatment groups, this ratio

TABLE II. EFFECT OF ACUTE APO, HALO, OR MOR INJECTION ON PLASMA PRL LEVELS (ng/ml) IN F344 CONTROL RATS, IN RATS TREATED WITH E_2 FOR 4 WEEKS AND IN RATS 4 WEEKS AFTER WITHDRAWAL OF CHRONIC E_2 TREATMENT

Group/ min	−40[a]	−20	+15	+30	+60	+90	Total DNA (μg)	PRL-cell DNA (μg)
APO								
(6) C	7.7 ± 2.0[b]	7.4 ± 1.0	6.8 ± 1.8	2.7 ± 0.4*	3.6 ± 0.6	3.0 ± 0.8*	104 ± 6	31 ± 2
(9) E	1960 ± 159	2247 ± 197	565 ± 155*	237 ± 32*	168 ± 13*	188 ± 22*	531 ± 44	318 ± 26
(10) WD	325 ± 50	354 ± 61	61 ± 9*	30 ± 4*	21 ± 3*	23 ± 3*	411 ± 15	170 ± 6
	−40	−20	+45	+90	+135	+180		
HALO								
(9) C	13.7 ± 5.2	13.1 ± 5.1	115.9 ± 17.9*	105.8 ± 13.8*	101.4 ± 15.7*	81.8 ± 12.0*	118 ± 4	35 ± 1
(9) E	847 ± 73	1132 ± 113	1847 ± 135*	2042 ± 139*	1959 ± 140*	1667 ± 140*	570 ± 38	341 ± 22
(10) WD	271 ± 52	257 ± 37	652 ± 74*	697 ± 78*	635 ± 84*	521 ± 81	371 ± 26	153 ± 11
	−40	−20	+15	+30	+60	+90		
MOR								
(6) C	8.9 ± 2.3	15.1 ± 2.6	162.5 ± 19.1*	98.2 ± 16.2*	32.7 ± 15.0	13.6 ± 2.5	102 ± 6	30 ± 2
(8) E	1503 ± 152	1581 ± 211	2539 ± 399	2741 ± 529*	2584 ± 395	2413 ± 361	562 ± 46	336 ± 28
(9) WD	207 ± 22	321 ± 35	547 ± 87*	694 ± 129*	659 ± 78*	581 ± 56*	358 ± 23	148 ± 10

Note. Also shown for each group is total AP DNA content (μg) and estimated DNA (μg) of PRL-secreting cells.

[a] Signifies time before or time following drug injection.

[b] $X \pm$ SEM; in OVX control rats (C), at the end of E_2 treatment (E), or after E_2 withdrawal (WD).

* $P \leq 0.05$ significantly different from −20-min preinjection sample.

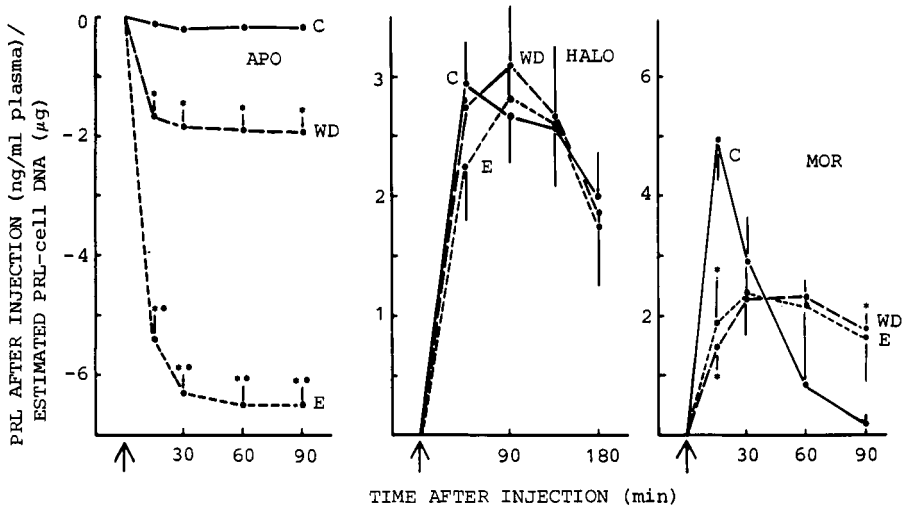

FIG. 1. Effects of acute administration of APO (left), HALO (center), and MOR (right) on stimulation or inhibition of PRL release in F344 control rats (C), in rats treated with E_2 for 4 weeks (E), and in rats 4 weeks after withdrawal of chronic E_2 treatment (WD). Data ($\bar{X}$ ± SEM of 6–10 animals/group) are expressed as the change in plasma PRL after drug injection (ng/ml) divided by the estimated portion of total AP DNA content which constitutes PRL-secreting cells (μg). Arrow indicates the time of drug injection. (* = $P \leq 0.05$ compared to C; ● = $P \leq 0.05$ compared to WD.)

is an estimate which is proportional to the change in PRL release per lactotroph in response to the drugs. This allows for evaluation of the PRL response to the drug without the bias of differing basal plasma PRL levels or the different number of lactotrophs among the treatment groups. The DNA attributed to the lactotrophs was calculated by first measuring total AP DNA content. For OVX F344 control and E_2-treated rats, Phelps and Hymer (24) counted the number of immunocytochemically identifiable lactotrophs after separation of AP cells from red blood cells on a Ficoll-Hypaque gradient. When these authors used an E_2-treatment similar to ours, they observed AP enlargements comparable to those reported here and 59.8% of the AP cells were identified as lactotrophs. Only 29.8% lactotrophs were found in OVX controls. In our experiment, the E_2-treated rats showed a 31% decline in mean AP DNA content (539 to 375 μg; see Table III) 4 weeks after the E_2-containing capsule was removed. Assuming that this decline was exclusively due to a loss of lactotrophs, the percentage loss of lactotrophs in rats after E_2 withdrawal for 4 weeks would be $0.598 \times 0.310 = 0.185$ or an 18.5% loss of lactotrophs (thus, the % of lactotrophs was $0.598 - 0.185 = 0.413$ or 41.3%). The estimate used for the portion of AP DNA content due

TABLE III. EFFECTS OF E_2 TREATMENT AND E_2 WITHDRAWAL ON ANTERIOR PITUITARY (AP) WEIGHT, DNA CONTENT, AND SERUM PRL AND LH LEVELS

	AP weight (mg)	AP DNA (μg)	AP DNA (μg/mg)	Serum PRL (ng/ml)	Serum LH (ng/ml)
1. OVX controls[a]	10.0 ± 0.3	112 ± 4	11.3 ± 0.4	26 ± 4	535 ± 77
2. E_2 treated	64.5 ± 4.7*	539 ± 37*	8.1 ± 0.3*	1695 ± 208*	29 ± 4*
3. E_2 withdrawal	30.0 ± 1.7*	375 ± 24*	12.3 ± 0.4	247 ± 26*	161 ± 22*

[a] $\bar{X}$ ± SEM of 11–12 animals/group; Group 1 was OVX for 8 weeks, Group 2 received E_2 for 4 weeks, Group 3 received E_2 for 4 weeks after which E_2 was withdrawn for 4 weeks.

* $P \leq 0.05$ compared to Group 1.

to the lactotrophs was 29.8% for OVX controls, 59.8% for E_2-treated rats and 41.3% for rats withdrawn from E_2 for 4 weeks. The total DNA content and the estimated portion due to lactotrophs is shown in Table II.

The inhibition of PRL per microgram PRL-cell DNA after acute APO administration (Fig. 1) was significantly greater in rats at the end of E_2 treatment (E) than in OVX controls (C) or in rats 4 weeks after E_2 removal (WD) ($P \leq 0.05$). Animals after removal of E_2 (WD) showed a greater PRL inhibition per microgram PRL-cell DNA after APO than OVX controls. Blockade of DA receptors by administration of HALO resulted in similar increases in plasma PRL in all three groups when the data were expressed per microgram PRL-cell DNA. Injection of MOR resulted in a rapid increase and then decline in PRL release in OVX control rats (C). The PRL rise in response to MOR was lower but more prolonged in E_2-treated rats (E) and in rats after withdrawal of E_2 (WD).

Since E_2-treated animals and animals after E_2 withdrawal exhibit a greatly enlarged AP, there is the possibility that the enlarged gland may impinge on blood flow between the median eminence and the AP. This could result in a smaller PRL response in the E_2-treated animals because of the limited blood flow in the portal vasculature, and reduce the passage of DA to the AP. To evaluate this possibility, the plasma GH response to acute MOR injection was also measured in the three treatment groups. Injection of 5 mg/kg MOR (Fig. 2) produced a significant increase in plasma GH in animals after E_2 removal for 4 weeks ($P \leq 0.05$), but MOR did not induce significant release of GH in control rats or in rats at the end of 4 weeks of E_2 treatment.

Table III shows that 4 weeks of E_2 treatment produced greater than a sixfold increase in AP weight and a 65-fold rise in serum PRL as compared to non-E_2-treated control rats. Both AP weight and serum PRL declined by the end of 4 weeks of E_2 withdrawal (Group 3), but remained elevated when compared with OVX controls ($P \leq 0.05$). AP DNA content increased from 112 μg in OVX controls to 539 μg after 4 weeks of E_2 treatment, and remained elevated (3×) above control values 4 weeks after E_2 withdrawal ($P \leq 0.05$). When DNA content was expressed per milligram AP tissue weight, E_2-treated rats showed reduced DNA content when compared with control values ($P \leq 0.05$). Four weeks of E_2 treatment significantly suppressed the LH response to OVX ($P \leq 0.05$). This suppression of LH release persisted 4 weeks after removal of the E_2.

Discussion. The results presented here confirm previous reports that chronic E_2 treatment depresses TIDA neuronal activity (13–15), and indicate that after removal of long-term E_2 treatment in F344 OVX rats, TIDA activity remains depressed. Attenuated TIDA neuronal activity is clearly indicated by the observation that at the end of E_2 treatment or even 4 weeks after E_2 removal, the electrically stimulated release of [^{3}H]DA from ME tissue was decreased when compared to rats not treated with E_2.

The plasma PRL response to APO, HALO, and MOR was expressed as the ratio of the change in plasma PRL after injection of the drug divided by an estimated portion of total AP DNA content which constitutes only the lactotrophs. In both E_2-treated groups, the estimates of PRL-cell DNA are presumed to be low. E_2-treatment results in low PRL content in the lactotrophs and therefore it is probable that a portion of the lactotrophs was not immunocytochemically identified as such. These possible low estimates of PRL-cell DNA are conservative in the sense that, after drug in-

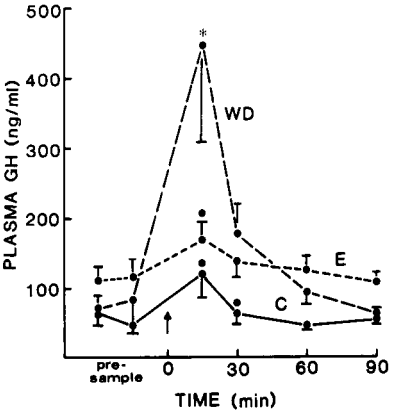

FIG. 2. Effect of MOR on the concentration of plasma GH in OVX (C), after E_2 withdrawal for 4 weeks (WD) and in E_2-treated (E) F344 rats. Arrow indicates the time of drug injection. ($X \pm$ SEM of 7–10 animals/group; * = $P \leq 0.05$ compared to preinjection GH level, ● = $P \leq 0.05$ compared to WD.)

jection, they allow for greater PRL responses in E_2-treated rats and rats after withdrawal from E_2. Decreased TIDA activity after E_2-treatment and after E_2-withdrawal is supported by the pharmacological evidence that acute administration of MOR to E_2-treated rats resulted in a slower and attenuated increase in plasma PRL when compared with OVX control rats. Chronic E_2 has been shown to elevate hypothalamic opiate binding sites (25) which might permit a larger increase in PRL in E_2-treated animals after MOR. However, E_2-treated rats showed a smaller rise in PRL compared to controls which further suggests a depressed TIDA function. Depression of TIDA neurons (e.g., by MOR) which have a low basal activity, i.e., E_2-treated rats and rats after E_2 withdrawal, increased PRL to a lesser degree than rats with a higher TIDA activity, i.e., controls. After HALO injection, all groups showed similar increases in circulating PRL levels, suggesting that a maximal blockade of DA action on the lactotrophs permitted a similar release of PRL per microgram PRL-cell DNA in all three treatment groups. APO produced a greater inhibition of PRL release in both E_2-treated groups as compared to controls, when the data are expressed as per lactotroph.

There is ample evidence suggesting that the circulating level of PRL is the major factor in regulating TIDA neuronal activity (2). During hyperprolactinemia associated with chronic E_2 treatment, TIDA neurons exhibit a decline in their responsiveness to administered PRL, due to a direct or indirect effect of E_2 on these neurons (15). Upon removal of E_2, the TIDA neurons appeared to regain responsiveness to PRL. However, in our experiments, after E_2 was removed for 4 weeks, the persistent hyperprolactinemia which was sufficient to suppress the LH response to OVX did not appear to stimulate TIDA function. The explanation may be that in the present study a higher dose of E_2 was administered for a longer duration of time. Also, we used the F344 rat strain, which is particularly sensitive to the action of E_2 on PRL secretion (9, 26). The effect of E_2 was comparatively greater on serum PRL levels and AP weights in the F344 rat, and may be responsible for the continued depression of TIDA function after E_2 removal.

There are potential problems in the evaluation of TIDA activity *in vivo* using lactotrophic response to DA agonists and antagonists, especially in animals with enlarged APs. A restricted blood flow in the portal vasculature or genesis of systemic arteries to the pituitary gland (27) as a result of AP growth could decrease the concentration of DA reaching the AP lactotrophs. This should result in a decrease in all the releasing/release-inhibiting factors of the hypothalamus that regulate AP function. The combination of decreased release of DA from TIDA neurons, together with the possible limited reduction in portal blood flow and development of new vascularization to the AP, could greatly reduce the inhibitory hypothalamic influence on PRL secretion. However, the reduced PRL release in E_2-treated animals after injection of MOR could be partially the result of less DA reaching the AP because of these physical factors and not be due to diminished TIDA activity. To evaluate this possibility, the ability of MOR to increase GH was measured in controls, in E_2-treated rats and in rats after E_2 withdrawal. MOR is known to increase GH by a central action on neurotransmitters, growth hormone releasing factor, and somatostatin (28). In the present study, MOR increased plasma GH to similar levels in control and E_2-treated animals; however, a fourfold greater increase in GH was observed in animals 4 weeks after withdrawal of E_2-treatment. If reduced portal blood flow and dilution of portal blood due to new vascularization were effective in reducing hypothalamic influence on the AP, the time course for the MOR-induced GH release among treatment groups should be similar to the time course of PRL release. However, the time course for GH release was very different from PRL, suggesting that the central nervous system mechanisms responsible for MOR-induced GH release were not affected by altered portal blood flow. This interpretation should be viewed with caution, since the influence of E_2 on the hypothalamic and AP mechanisms effecting GH release is not well understood at present (28).

A number of mechanisms may be responsible for the persistence of reduced TIDA activity after removal of E_2. Long-term E_2 treatment produces a characteristic hypothalamic arcuate nucleus lesion and functional endocrine deficits associated with it (11, 12). To

what extent E_2-induced arcuate nucleus lesions involve TIDA neurons is unknown, although it has been shown that E_2 is concentrated in tyrosine-hydroxylase containing neurons of the arcuate nucleus (29). Whether 4 weeks of E_2 treatment in OVX F344 rats is sufficient to produce an arcuate nucleus lesion is not known. Damage to TIDA neurons has been suggested by findings of decreased DA fluorescence in the arcuate nucleus of female rats treated chronically with E_2 (5). If long-term E_2 treatment damages TIDA neurons in F344 rats, the decline in TIDA function should persist after removal of E_2. Long-term hyperprolactinemia induced by transplant of a PRL-secreting tumor also has been reported to diminish DA fluorescence in the arcuate nucleus and to decrease TIDA function. Thus, possible TIDA neuronal degeneration could be due in part to chronic hyperprolactinemia (5, 6). Physical compression of the medial basal hypothalamus by AP enlargement also may contribute to these potentially irreversible effects of E_2 on TIDA neurons. After only 4 weeks of E_2-treatment in F344 rats, it was observed that AP size was sufficient to induce compression of the medial basal hypothalamus. Compression was still observed, although to a lesser degree, even after E_2 had been removed for 4 weeks. Thus, physical compression, as well as high E_2 or prolactinemia, may induce functional damage to TIDA neurons. This possibility is currently being investigated.

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