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Bromocryptine Prevents the Decline in Tuberoinfundibular Neuronal Release of Dopamine after Removal of Chronic Estrogen Treatment (42595)

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Abstract. Prolonged exposure to estradiol 17-β (E_2) in rats has been shown to decrease dopamine (DA) synthesis in and release from tuberoinfundibular dopaminergic (TIDA) neurons in Fischer 344 rats. The objective of the present study was to determine whether inhibition of the E_2-induced increase in anterior pituitary (AP) weight and prolactin (PRL) secretion by concomitant administration of the dopaminergic agonist, bromocryptine, could prevent the decrease in TIDA neuronal function produced by chronic E_2 administration. TIDA neuronal function was evaluated by *in vitro* superfusion and electrical stimulation of median eminence (ME) tissue after allowing for accumulation of [^{3}H]dopamine (DA). The effect of chronic E_2 and/or bromocryptine treatment on catecholamine content in tuberohypophyseal neurons in the neurointermediate lobe was also measured to determine whether increased pituitary size possibly damaged the tuberohypophyseal neurons. Treatment with E_2 for 30 days significantly increased AP weight, serum PRL concentration, and AP PRL and DNA content over values in non-E_2-treated controls. When bromocryptine was injected daily during E_2 treatment, bromocryptine completely inhibited the E_2-induced increase in serum PRL and AP DNA content, and AP weight was only moderately increased. The evoked release of ^{3}H at the end of the 30-day E_2 treatment was reduced during electrical stimulation and there was no augmented release of ^{3}H from the ME tissue after 10 μM nomifensine infusion in E_2-treated rats and in rats given both bromocryptine and E_2. However, neurointermediate lobe DA content was diminished only in E_2-treated rats and not in animals given bromocryptine together with E_2. When all treatments were discontinued for 30 days, animals previously given only E_2 showed sustained increases in AP weight, serum PRL levels, and AP PRL and DNA content, but reduced stimulation-evoked release of ^{3}H, absence of response to nomifensine, and reduced neurointermediate lobe DA and norepinephrine content when compared with values in non-E^2-treated controls. After withdrawal of E_2 treatment for 30 days, animals previously given bromocryptine and E_2 together were not different from control animals in any of the parameters measured. These results suggest that the decline in TIDA neuronal release of DA induced by chronic E_2 treatment was at least partly exerted via the marked hyperprolactinemia and/or by compression of the medial basal hypothalamus by the enlarged AP. © 1987 Society for Experimental Biology and Medicine.

High levels of circulating estradiol maintained for prolonged periods in rats can decrease tuberoinfundibular dopaminergic (TIDA) neuronal activity (1–6). Demarest *et al.* (4) observed in Long–Evans rats that the depression of dopamine (DA) synthesis in TIDA neurons after 18 days of estradiol 17-β (E_2) treatment was reversed when the E_2 was removed from the animals. Others (7) have shown that after termination of chronic treatment in male Fischer (F) 344 rats with diethylstilbestrol, there was a rebound increase in TIDA neuronal synthesis to double that of control levels (23). However, we (6, 8) have observed a decreased ability of TIDA neurons to release DA *in vitro* even 6 months after removal of more prolonged (4 weeks) E_2 treatment in female F344 rats. The anterior pituitary (AP) of the F344 ovariectomized (OVX) rat is known to be very sensitive to E_2 treatment, and increases in AP weight, size, and prolactin (PRL) secretion proceed much faster than in other rat strains (9, 10).

In this study, we have attempted to elucidate the mechanism(s) by which chronic E_2 treatment in female F344 rats results in deficiencies in the release of DA from TIDA neurons. Bromocryptine was administered to determine whether it could inhibit the effects of chronic E_2 treatment on AP growth, hyperprolactin-

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emia, and the decline in release of DA from TIDA neurons.

Materials and Methods. *Animals and treatment.* Three-month-old female Fischer (F) 344 rats were purchased from Harlan Industries (Indianapolis, IN), housed under controlled light and temperature (lights on 0500–1900 hr, 26 ± 2°C), and given free access to rat chow (Ralston Purina, St. Louis, MO) and tap water. All animals were OVX immediately prior to treatment. Half the rats were divided into four groups: (i) OVX controls implanted with an empty Silastic capsule (Dow Corning, Midland, MI) sc for 30 days and given daily vehicle injections, (ii) rats given daily bromocryptine injections for 30 days (Sandoz, Hanover, NJ; 3 mg/kg, sc, 50% ethanol in 0.85% NaCl vehicle) and implanted with an empty capsule, (iii) rats given an E_2-filled capsule (10 mm in length, 0.078 i.d. × 0.125 o.d.) and daily vehicle injections, and (iv) rats given bromocryptine daily together with an E_2-filled capsule. The other half of the animals were treated the same as above except that treatment was discontinued after 30 days, and the rats were left untreated for 30 more days before acute experiments were performed. All animals of the first four groups were sacrificed 24 hr after the last bromocryptine or vehicle injection.

Release of ^3H from median eminence in vitro after accumulation of ^3H-DA. In each group, the ability of TIDA neurons to release DA was measured using an *in vitro* superfusion technique after allowing for accumulation of [^{3}H]DA into median eminence (ME) tissue, as previously described (11, 6). The release of ^{3}H was expressed as a fractional rate constant (11), and the tissue was electrically stimulated for 15 sec and 50 and 80 min after the beginning of superfusion. The stimulation-evoked release of ^{3}H was calculated by subtracting the sum of the two fractional rate constants prior to stimulation (baseline) from the sum of the two fractional rate constants after stimulation (peak area).

At 60 min after the beginning of superfusion, nomifensine maleate (10 μM; Hoechst-Roussel, Somerville, NJ), was added to the medium to evaluate ^{3}H release from the ME tissue after inhibition of DA reuptake (12, 13). Experiments were performed starting at 1100 hr. Animals were decapitated, blood was collected, and serum was separated and frozen for PRL radioimmunoassay. ME tissue was dissected for superfusion. The AP was separated from the neurointermediate lobe, weighed, homogenized in 2 ml of 0.01 *M* phosphate-buffered saline (pH 7.6), and frozen for later measurement of AP PRL and DNA content.

Assays. Serum and AP PRL were measured by radioimmunoassay (RIA) with reagents provided by the National Pituitary Agency of the NIADDK, except that rabbit anti-rat PRL was provided by Dr. C. L. Chen (University of Florida). IgGSorb (Enzyme Center, Boston, MA) was used to separate bound from free hormone. Serum and AP PRL were expressed in terms of rPRL-RP-3. The intra- and inter-assay coefficients of variation were 6.8 and 10.8%, respectively. The minimum detectable dose for PRL was 0.09 ng/tube.

The catecholamine assay was a modification of the radioenzymatic method of Cheng and Wooten (14). Neurointermediate lobes were dissected and homogenized in 250 μl of 0.1 *N* $HClO_4$ containing 5 m*M* glutathione (Sigma Chemical Co., St. Louis, MO). The homogenate was centrifuged, and the supernatant was stored frozen at −40°C until assay. The DA and norepinephrine (NE) contents were expressed as nanograms of catecholamine per milligram of neurointermediate lobe protein as measured by the Bio-Rad assay (15), and expressed as a percentage of the control values, either at the end of treatment or 30 days after treatment was terminated. AP DNA content was measured using the method of Burton (16).

Statistical analysis. The AP weight, serum and AP PRL concentrations, and AP DNA content revealed a significant F_{max} test for heterogeneity of variance. The data were transformed logarithmically before performing analysis of variance and the Student–Newman–Keuls multiple-comparison test. Data expressed as a ratio were analyzed by the Wilcoxson–Mann–Whitney test. The contents of DA and NE in the neurointermediate lobe were expressed as a percentage of the control values and the data were treated by analysis of variance. $P \leqslant 0.05$ was chosen as the level of significance.

Results. Thirty days of E_2 treatment of OVX F344 rats (E; open bars) increased AP

weight 10-fold, serum PRL 140-fold, AP PRL content 15-fold, and AP DNA content about 6-fold as compared to values in non-E_2-treated controls (C, open bars, Fig. 1). Bromocryptine given alone for 30 days (B, open bars) reduced serum PRL and AP PRL content but did not alter AP weight and DNA content when compared to control values. When bromocryptine was injected daily together with E_2 (BE, open bars), it completely inhibited the E_2-induced increase in serum PRL and AP DNA content and resulted in only a modest increase in AP weight as compared values in OVX controls.

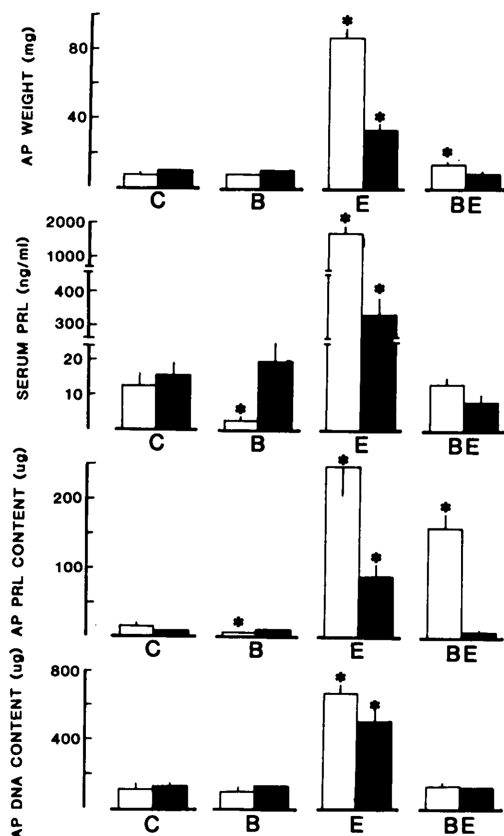

FIG. 1. Effects of 30 days of vehicle injections (C), bromocryptine (B), estradiol-17-β (E), or bromocryptine and estradiol treatment (BE; open bars) or 30 days after withdrawal of these treatments (solid bars) in OVX F344 rats on AP weight, serum prolactin concentration, and AP prolactin and DNA content. ($\bar{X}$ ± SEM, n = 12). Note that estradiol-induced increases in the parameters were reduced or prevented by concomitant bromocryptine treatment. *P < 0.05 compared to the appropriate (C) values.

However, bromocryptine did not significantly alter the E_2-induced increase in AP PRL content. Thirty days after E_2 withdrawal (E, solid bars), AP weight, serum PRL levels, and AP PRL and DNA content fell markedly when compared to values at the end of E_2 treatment (E, open bars), but remained higher than in the control animals (C, solid bars). The decreases observed during treatment with bromocryptine alone were not maintained after drug removal. Bromocryptine administered during E_2 treatment (BE, open bars) completely prevented the E_2-induced increases in serum PRL and AP DNA content, with only a modest, but significant, increase in AP weight. Thirty days after termination of treatment, animals which received bromocryptine and E_2 were not different from controls for any of the parameters shown in Fig. 1.

Figure 2 shows the stimulation-evoked release of ^{3}H after accumulation of [^{3}H]DA in the ME in the eight experimental groups in the absence (open or solid bars) and presence (stippled bars) of 10 μM nomifensine. Stimulation-evoked release of ^{3}H in the absence of nomifensine (open bars, Fig. 2) was significantly reduced in both E_2-treated groups (E and BE, open bars) when compared to controls (C, open bar), and there was a trend for reduced stimulation-evoked release in rats given only bromocrypine (B). The controls (C, open bars) and rats given bromocryptine alone (B, open bar) exhibited an increased stimulation-evoked release of ^{3}H in the presence (stippled bar), as compared to the absence (open bars) of 10 μM nomifensine. However, neither E_2-treated group (E and BE, open bars) responded significantly to nomifensine. Thirty days after withdrawal of the treatments, only animals after withdrawal of E_2 (E, solid bar) showed a reduced stimulation-evoked release of ^{3}H and no significant response to nomifensine compared to controls (C, solid bar). The reduced stimulation-evoked release observed in the presence of combined bromocryptine and E_2 treatment (BE, open bar) did not occur 30 days after removal of the combined treatment (BE, solid bar). In fact, bromocryptine and E_2-treated animals responded to nomifensine infusion similarly to controls at the end of the 30-day withdrawal period.

Neurointermediate lobe DA and NE content for each experimental group are shown

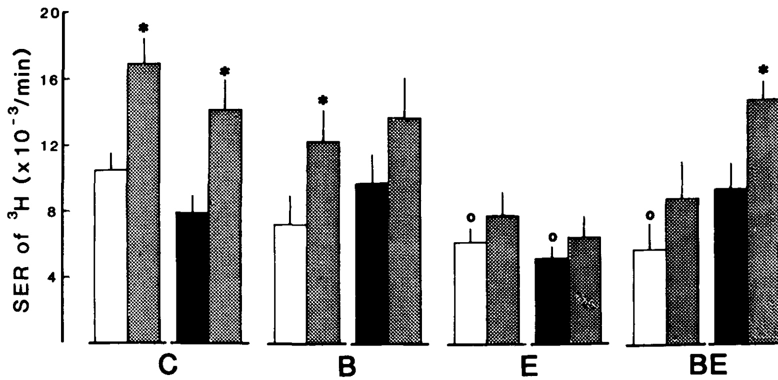

FIG. 2. Stimulation-evoked release (SER) of ^3H ($\bar{X} \pm$ SEM, $n = 6$) before (open and closed bars) and during infusion of 10 μM nomifensine (stippled bars) in bromocryptine and/or estradiol-17-β-treated animals or 30 days after withdrawal of treatment in OVX F344 rats. Open bars represent data from experiments at the end of the 30-day treatment, whereas closed bars represent data gathered from animals 30 days after termination of treatment. Note that in both estradiol-treated groups (E and BE, open bars) stimulation-evoked release of ^3H was reduced as compared to controls (C, open bar) and response to nomifensine (stippled bars) was absent. However, after withdrawal of treatment, stimulation-evoked release was reduced in animals treated with estradiol only (E, solid bar), and not in animals treated with both estradiol and bromocryptine (BE, solid bar). °$P < 0.05$ compared to C values; *$P < 0.05$ compared to evoked release in absence of nomifensine.

in Fig. 3. Neurointermediate lobe DA content was reduced about 50% in animals given only E_2 (E, open bar), but not in rats given E_2 and bromocryptine (BE, open bar), when compared to values in OVX control (C, open bars). After discontinuing all treatments for 30 days, neurointermediate lobe DA and NE content were significantly reduced only in animals 30 days after removal of E_2 (E, solid bar), compared to non-E_2-treated controls (C, solid bar).

It was observed upon autopsy of the rats at the end of E_2 treatment for 30 days that the large AP (87.7 ± 4.5 mg vs 8.5 ± 0.7 mg in controls) compressed the basal hypothalamus. Although AP weight and size was reduced 30 days after removal of E_2 treatment (34.9 ± 2.3 mg), there was persistent hypothalamic compression by the AP. In both of these groups which exhibited hypothalamic compression by the AP, distortion of the third ventricle was visible after excision of the ME, but the pituitary stalk remained at least grossly intact. The minimal increase in AP weight that occurred after 30 days of E_2 and bromocryptine treatment (15.4 ± 0.4 mg) was not large enough to compress the hypothalamus.

Discussion. The present results confirm that chronically elevated levels of circulating E_2 in F344 rats can produce a long-lasting decline in DA release from TIDA neurons (6, 8). They also show that after the withdrawal of treatment this DA deficiency can be prevented by simultaneous administration of the dopaminergic agonist, bromocryptine. Bromocryptine also inhibited the E_2-induced increases in AP weight and PRL secretion. Although bromocryptine did not prevent the decline in the release of DA from TIDA neurons in the presence of E_2, it was able to reverse the deficits induced by E_2 after E_2 treatment was discontinued. Since bromocryptine prevented the increases in AP weight and PRL secretion in the E_2-treated animals, it appears that the large increase in AP weight and/or hyperprolactinemia are mainly responsible for the decline in TIDA neuronal release of DA after termination of E_2 treatment, rather than a direct action of E_2 on TIDA neurons. Others have reported that chronic E_2 treatment can result in a hyperactive glial response in the arcuate nucleus suggestive of neuronal degeneration (17,18). However, it is not known whether the E_2-induced lesion involves TIDA neurons.

In the presence of elevated E_2 levels, the stimulation-evoked release of ^3H was reduced after [^3H]DA accumulation, and the responsiveness to nomifensine was absent when bromocryptine was administered together with

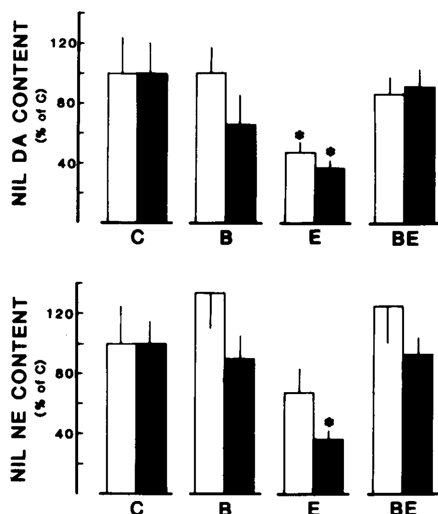

FIG. 3. Neurointermediate lobe dopamine (DA) and norepinephrine (NE) content ($\bar{X} \pm$ SEM, $n = 12$) expressed as percentage of (C) in bromocryptine and/or estadiol-17-β-treated (open bars) or 30 days after withdrawal of treatment (closed bars) in OVX F344 rats. Note that estradiol treatment alone (E) reduced neurointermediate lobe DA and NE content before (open bars) and after (solid bars) withdrawal of treatment. *$P < 0.05$.

E_2. This indicates that even when PRL levels are low and AP size is comparable to that in untreated animals, E_2 can still significantly decrease TIDA neuronal activity. After injection of [^{3}H]E_2, E_2-concentrating perikarya were localized in the arcuate nucleus (as indicated by autoradiography), within neurons that contained immunoreactive tyrosine hydroxylase (19). Implantation of E_2 in the ME resulted in marked increases in serum PRL 25 days later (20). Moreover, short-term E_2 treatment increased DA turnover in hypophysectomized rats (21). Many other studies have demonstrated a role for E_2 in regulating TIDA neuronal function, but suggested that its action was exerted indirectly, via PRL (22–24). However, our results indicate that chronic E_2 may have a direct physiological action in depressing the release of DA from TIDA neurons. Bromocryptine alone has previously been shown to reduce either the release or the synthesis of DA in TIDA neurons either by specifically binding to dopaminergic autoreceptor sites on terminals of TIDA neurons (11) or by diminishing circulating PRL levels and thereby lessening the short-loop feedback of PRL on DA neurons (12). Since bromocryptine alone did not significantly reduce the release of DA from TIDA neurons in OVX F344 rats, the major factor responsible for depressing TIDA neuronal release of DA after combined bromocryptine and E_2 treatment was probably the increased E_2 levels.

E_2 administration for 30 days in F344 OVX rats resulted in AP weights over 80 mg and serum PRL levels of about 1500 ng/ml. It was observed that the large AP compressed the basal hypothalamus and may have damaged neurons of the arcuate nucleus. This receives support from the observation that DA content was decreased in the neurointermediate lobe after treatment with E_2 alone, but not after E_2 and bromocryptine treatment. Tuberohypophyseal dopaminergic neurons also have their origins in the arcuate nucleus (26), and decreased DA content in the terminals of these neurons is suggestive of damage, since short-term E_2 or PRL treatment have not been shown to alter (4, 24) or increase (27) tuberohypophyseal DA content.

It is interesting to speculate whether in chronically E_2-treated rats with large APs there is reduced blood flow to the arcuate nucleus and ME. If this occurs, reduced O_2 delivery to the arcuate nucleus and ME for prolonged periods could result in decreased TIDA neuronal synthesis, since O_2 is a required substrate for the action of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis (28). Tyrosine hydroxylase has a relatively high K_m for O_2 (29) and DA synthesis may decrease with even a moderate acute decline in O_2 delivery (29, 30). Although chronic hypoxia may lead to an adaptive increase in tyrosine hydroxylase activity (30), E_2 treatment for 7 days was reported to depress tyrosine hydroxylase activity in medial basal hypothalamic tissue. It was suggested that the major site of E_2 action on tyrosine hydroxylase was on TIDA neurons (31). Therefore, decreased O_2 delivery and a direct effect of E_2 on tyrosine hydroxylase, together with the physical compression on the ME from the enlarged AP, may be largely responsible for the functional deficiencies in TIDA neurons that develop after chronic E_2 treatment.

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