Comparative Effects of Tamoxifen and Bromocriptine on Prolactin and Pituitary Weight in Estradiol-Treated Male Rats

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Male ACI rats were treated with estradiol to induce hyperprolactinemia and pituitary hypertrophy and hyperplasia. Animals received estradiol alone or with tamoxifen or bromocriptine for 4, 8, or 12 weeks. Estradiol treatment resulted in time-dependent increases in pituitary wet weight and serum prolactin concentrations. Tamoxifen completely blocked the increase in both variables; bromocriptine decreased but did not prevent time-dependent increases. Animals were also treated for 8 weeks with estradiol alone, followed by 4 weeks with estradiol and tamoxifen or bromocriptine. Neither compound reversed the hyperprolactinemia, although the pituitary wet weight of animals treated with bromocriptine was slightly but significantly reduced. These findings suggest that in this model if treatment is initiated simultaneously with estrogen stimulation, tamoxifen is more effective than bromocriptine at the doses studied; and, if therapy is initiated subsequent to the establishment of estrogen-induced hyperprolactinemia and pituitary hyperplasia, bromocriptine is more effective than tamoxifen at the doses studied.

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THE PHYSIOLOGIC AND PHARMACOLOGIC effects of estrogen on prolactin secretion in mammals are well known. During pregnancy, estradiol induces an increase in prolactin in preparation for lactation. However, estrogens may also cause abnormal hypertrophy and hyperplasia of mammotrophs. Treatment of women with estrogen-containing oral contraceptives may be associated with hyperprolactinemia and amenorrhea and, in some cases, with the development of pituitary adenomas.[1] Induction of hyperprolactinemia, pituitary hypertrophy and hyperplasia, and pituitary tumors has been demonstrated in rats and mice[2–5]; the ACI strain of rats appears to be particularly susceptible to these effects of estrogens.[4,5] However, the molecular mechanisms by which estrogens exert these effects on mammotrophs are largely unknown.

Dopamine is a physiologic inhibitor of prolactin secretion. Estradiol appears to be a potent antidopaminergic agent *in vivo*.[6] Bromocriptine (2-bromo-α-ergocryptine, a dopamine agonist) has been used to examine the interaction between estrogen and dopamine in controlling prolactin secretion *in vivo*. In Sprague-Dawley rats treated with a single dose of estrogen, bromocriptine decreased serum prolactin concentration and inhibited the estrogen-induced increase in pituitary wet weight and mitotic activity.[7] Clinically, bromocriptine has been used successfully to treat hyperprolactinemia[1] and to reduce the size of prolactinomas.[8,9] However, bromocriptine was not effective in suppressing serum prolactin concentrations or in reducing the size of transplantable pituitary tumors MtTW15 and 7315a carried in female rats.[10,11]

The antiestrogenic effects of tamoxifen on prolactin synthesis and secretion have also been examined. Treatment with tamoxifen alone does not affect serum prolactin concentrations in untreated female rats, although the synthesis of prolactin decreased.[12] The increased serum prolactin level seen after 7 days of treatment with estradiol was partially blocked by simultaneous treatment with tamoxifen.[13] Nagy and coworkers[12] and de Quijada and coworkers[11] reported suppression of growth of the estrogen-dependent transplantable pituitary tumor 7315a by tamoxifen; the effects of tamoxifen on serum prolactin concentrations in this model were dose-dependent and time-dependent.[11,12] In eight patients with invasive prolactin-secreting pituitary adenomas, tamoxifen treatment

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lowered serum prolactin levels and potentiated the effects of bromocriptine.[14]

This study was designed to compare the effects of bromocriptine and tamoxifen on pituitary wet weights and serum prolactin concentrations in animals chronically stimulated with estrogen. We report here changes in these parameters in male ACI rats continuously exposed to estradiol when treatment with these drugs was initiated simultaneously with estradiol treatment, and when treatment with these drugs was initiated following a period of estradiol stimulation.

Materials and Methods

Animals

Male rats of the ACI strain (Harlan Industries, Cumberland, IN) were used at 50 to 55 days of age. Animals were maintained under a controlled lighting cycle (lights on 0800–2000 hour) with food and water *ad libitum*. Body weights were obtained 3 times per week. At the termination of the experiment, the animals were killed by decapitation. Trunk blood was collected and serum was stored at −20°C. The whole pituitary gland from each animal was removed, weighed, and frozen within 2 minutes of death.

Estrogen Treatment

Estradiol was obtained from Sigma Chemical Co. (St. Louis, MO) and placed in 3 cm silastic capsules (Dow Corning; id 1.98 mm, od 3.18 mm) as described by Karsch and associates.[15] The capsules were implanted subcutaneously while animals were maintained under light ether anesthesia; capsules were replaced at 4 week intervals to minimize fibrous connective tissue encapsulation and to ensure continuing high-serum estradiol levels. The implants were removed 72 hours before death.

Drug Treatment

Subcutaneous injections of bromocriptine, tamoxifen, or vehicles were given daily. Bromocriptine mesylate (Parlodel, Sandoz Pharmaceuticals, East Hanover, NJ) was suspended in sterile 0.9% NaCl to give a final concentration of 1 mg/ml. Each animal received 0.2 ml (267 nmoles bromocriptine or approximately 900 μg/kg body weight) daily. Tamoxifen citrate (Nolvadex, Stuart Pharmaceuticals, Wilmington, DE) was suspended in propylene glycol containing 2.5% ethanol at a final concentration of 0.5 mg/ml. Each animal received 0.2 ml (167 nmoles tamoxifen or approximately 300 μg/kg body weight) daily. Animals implanted with estradiol-containing capsules but receiving no additional drugs and animals implanted with empty capsules were injected daily with both vehicles. Animals receiving one drug were given daily injections of the other vehicle. Animals in the control group received no injections, and were handled only for weighing.

The effects of tamoxifen and bromocriptine on estradiol-induced pituitary hyperplasia were examined in two treatment protocols. In the first experiment, the effectiveness of tamoxifen and bromocriptine in preventing the induction of hyperprolactinemia and pituitary hypertrophy and hyperplasia by estradiol was compared. Groups of eight animals were treated for 4, 8, or 12 weeks with estradiol alone, with estradiol and tamoxifen, or with estradiol and bromocriptine. To determine the effects of the experimental manipulations on serum prolactin levels and pituitary wet weights, eight animals were implanted with empty capsules, and received injections of both vehicles. A fifth group of eight animals was untreated, and served as a control.

To compare the effectiveness of treatment with bromocriptine or tamoxifen after estradiol-induced hyperprolactinemia and pituitary hyperplasia are established, animals were treated according to the following protocol: two groups of eight animals were implanted with estradiol-containing capsules and received daily injections of both vehicles for eight weeks. During the 4 subsequent weeks, the estradiol capsules remained in place, and the animals received either bromocriptine (267 nmoles daily) or tamoxifen (167 nmoles daily).

Radioimmunoassays

Serum estradiol was monitored in animals implanted with capsules identical to those used in the study groups. Serum samples were collected weekly from the tail vein, and estradiol was measured by specific radioimmunoassay.[16] Serum prolactin was measured by a double antibody radioimmunoassay for rat prolactin using reagents supplied by the National Pituitary Agency, National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD), Bethesda, MD. Prolactin values are expressed as NIAMDD rat prolactin RP-1. All samples were included in the same assay.

Statistical Analysis

All data are expressed as mean ± standard error of the mean. An initial two-way analysis of variance was performed to detect differences among the means for duration of treatment and for treatment protocol. Newman-Keuls statistics were then employed to detect differences between individual means. The effects of time were also analyzed by least-squares linear regression; these data are expressed as slope of the regression line (m) and correlation coefficient (r). One-way analysis of variance was performed on data from the rats treated for 12 weeks to determine the effects of the delayed treatment groups. This was fol-

lowed by Newman-Keuls statistics to compare individual means. Student's t test was used to compare delayed-treatment groups with the 8-week-estradiol-treatment group. Differences with $P < 0.05$ were considered to be significant.

Results

Estrogen Treatment

Serum estradiol concentrations in rats (n = 6) implanted with capsules containing estradiol ranged from 5 to 20 ng/ml. There was no trend in estradiol concentration with respect to the length of time the capsule remained in the animal. When the animals were killed, serum estradiol levels were 2 to 3 ng/ml. The serum concentration of estradiol in untreated male ACI rats ranged from 10 to 20 pg/ml (n = 4).

Animals receiving estradiol alone or in combination with bromocriptine or tamoxifen gained weight more slowly than either treated or untreated control animals during the first 8 weeks of the study. However, the body-weight differences among treatment groups were less apparent at 12 weeks. At this time, the body weights of animals receiving estradiol alone were not significantly different from those of either control group, although the body weights of animals receiving estradiol and bromocriptine or tamoxifen were still significantly depressed.

Pituitary Wet Weights

The effects of treatment regimen and length of treatment on pituitary wet weights are shown in Figure 1. Two-way analysis of variance revealed significant effects of both time and treatment. The pituitary weights of rats treated with estradiol alone increased significantly with length of treatment (m = 5.09; r = 0.696). At all time points studied, the pituitary weights of estradiol-treated rats were significantly greater than those of rats bearing empty capsules or of untreated animals.

Tamoxifen treatment completely blocked the estradiol-induced increase in pituitary wet weight. The pituitary weights of animals receiving estradiol and tamoxifen were not significantly different at any time point from those of untreated rats, and did not increase with length of treatment. Pituitary wet weights of rats treated with estradiol and tamoxifen were not significantly different at 8 or 12 weeks from those of animals bearing empty capsules; however, at 4 weeks tamoxifen-treated rats had larger pituitaries than rats with empty capsules.

Bromocriptine significantly inhibited the estradiol-induced increase in pituitary wet weight at all time points, but it did not prevent a significant, time-dependent increase in pituitary wet weight (m = 0.63; r = 0.786). When rats treated with estradiol and bromocriptine were

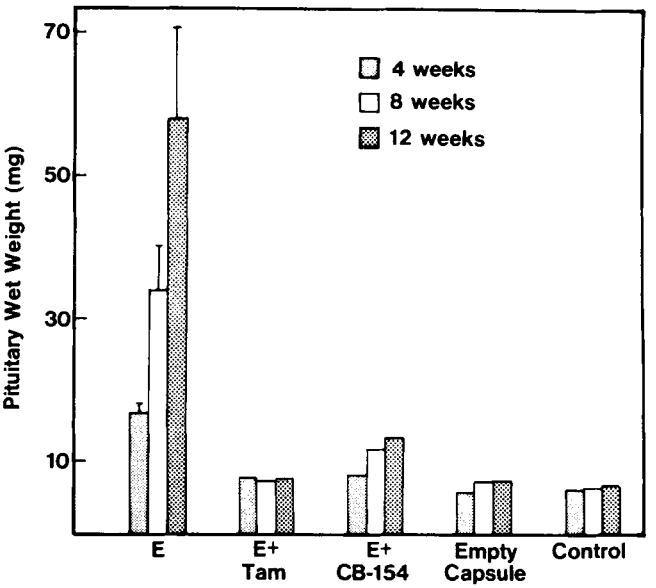

FIG. 1. Pituitary wet weights (mg) of rats sacrificed after 4, 8, or 12 weeks treatment with estradiol (E), estradiol and tamoxifen (E + Tam), estradiol and bromocriptine (E + CB-154), empty implants and vehicle injections (empty capsule), or untreated animals (control). Data are expressed as mean ±SE or mean (when SE < 1 mg) for each group of 6–8 animals.

compared with those treated with estradiol and tamoxifen, pituitary weights were not different at 4 weeks. However, the pituitaries of bromocriptine-treated rats were significantly larger than those of tamoxifen-treated rats at both 8 and 12 weeks. At all time points the pituitary weights of animals treated with estradiol and bromocriptine were significantly greater than those either of untreated rats or of rats bearing empty capsules.

Pituitary weights of rats bearing empty capsules increased slightly but significantly with time (m = 0.17; r = 0.498), while those of untreated rats did not increase with time. Comparison of means of pituitary weights for individual groups indicated no significant difference at any time between animals bearing empty capsules and untreated animals.

Serum Prolactin

The effects of treatment and time on serum prolactin levels are shown in Figure 2. Two-way analysis of variance revealed significant differences with respect to treatment and time. Prolactin levels of rats treated with estradiol alone increased in a nonlinear, time-dependent manner. As with pituitary weights, serum prolactin levels in these animals were significantly greater for each treatment duration than those in untreated rats or in rats treated with estradiol and tamoxifen or with estradiol and bromocriptine.

Serum prolactin concentrations in rats treated simultaneously with estradiol and tamoxifen were significantly

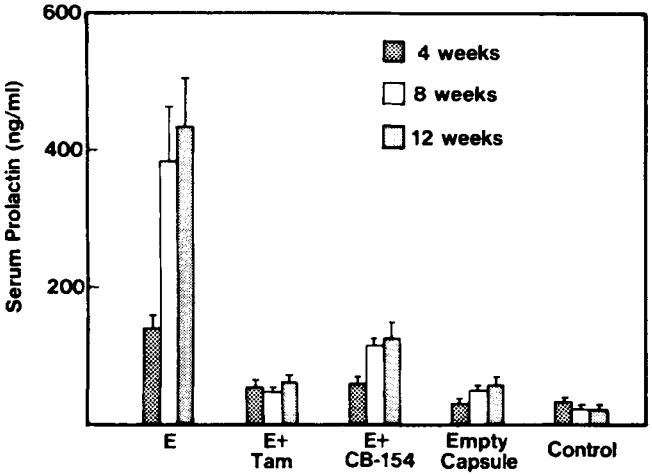

FIG. 2. Serum prolactin levels (ng/ml) in rats sacrificed after 4, 8, or 12 weeks treatment with estradiol (E), estradiol and tamoxifen (E + Tam), estradiol and bromocriptine (E + CB-154), empty implants and vehicle injections (empty capsule), or untreated animals (control). Data are expressed as mean ±SE for each group of 6–8 animals.

lower than those in rats treated with estradiol alone, and did not increase with time. There was no significant difference between these levels and those at 8 or 12 weeks in animals bearing empty capsules; at 4 weeks serum prolactin concentrations were slightly but significantly higher in tamoxifen-treated animals than in rats bearing empty capsules. Serum prolactin concentrations in animals treated with estradiol and tamoxifen were significantly higher than those in untreated rats for each duration of treatment.

Although simultaneous treatment with bromocriptine significantly inhibited the magnitude of the estradiol-induced increase in serum prolactin levels, it did not prevent a time-dependent increase in serum prolactin (m = 6.87; r = 0.464). Bromocriptine and tamoxifen were equally potent in inhibiting the increase in serum prolactin seen at 4 weeks, but at 8 and 12 weeks serum prolactin concentrations in animals treated with estradiol and bromocriptine were significantly higher than those in rats treated with estradiol and tamoxifen. Serum prolactin levels in rats treated with estradiol and bromocriptine were also significantly higher at all times than those in animals bearing empty capsules or in untreated animals.

Serum prolactin levels in rats bearing empty capsules increased slightly but significantly with time (m = 3.48; r = 0.482), whereas prolactin levels of untreated rats did not change with time. Serum prolactin in animals bearing empty capsules was significantly higher than that in untreated animals at 8 and 12 weeks.

Figure 3 compares the effects of tamoxifen and bromocriptine on pituitary wet weights and serum prolactin concentrations in animals stimulated with estradiol for 8 weeks before beginning treatment with tamoxifen or bromocriptine. Serum prolactin levels in these animals were not significantly different from those in animals exposed to estradiol alone for 8 weeks, but were significantly lower than those in animals exposed to estradiol alone for 12 weeks. Pituitary wet weights in animals treated with tamoxifen in this protocol were not significantly different from those in animals treated for 8 weeks with estradiol alone, but were significantly lower than those in rats treated for 12 weeks with estradiol alone. Bromocriptine treatment in this protocol resulted in pituitary wet weights which were not only significantly lower than those from animals receiving estradiol for 12 weeks, but were also significantly lower than those from animals receiving estradiol for 8 weeks.

Linear regression analysis of pituitary wet weight and serum prolactin in rats treated with estradiol only revealed a significant positive correlation between the two parameters (m = 8.25; r = 0.905). A similar correlation was not seen in rats treated with estradiol and bromocriptine, although both parameters increased with the length of treatment.

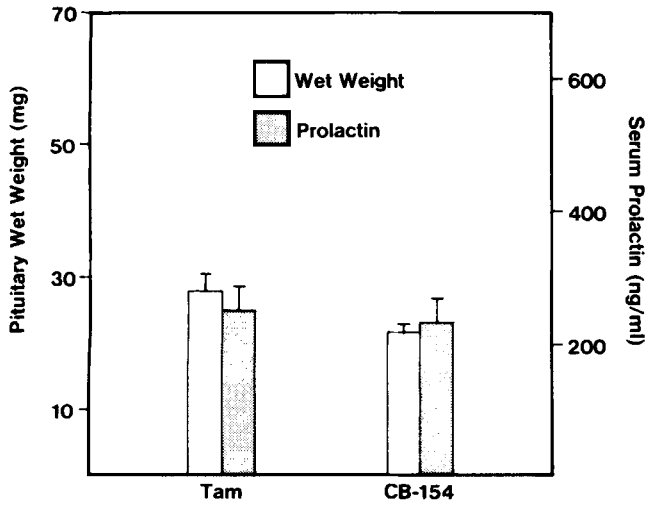

FIG. 3. Pituitary wet weights (mg) and serum prolactin levels (ng/ml) in rats treated with estradiol alone for 8 weeks followed by 4 weeks treatment with estradiol and tamoxifen (Tam) or estradiol and bromocriptine (CB-154) until death. Data are expressed as mean ±SE for each group of 6–8 animals.

Discussion

Long-term treatment of male ACI rats with estradiol resulted in a marked increase in pituitary wet weight and serum prolactin. These results are similar to those reported by other investigators using a variety of both acute and chronic models.[2–5,7]

Simultaneous treatment with tamoxifen at this dose completely blocked the estradiol-induced increases in pituitary wet weights and serum prolactin levels. Neither parameter was significantly different from that in either control group with the exception of the 4-week point. At this time a slight but significant increase in serum prolactin

and pituitary wet weight was noticed in rats treated with estradiol and tamoxifen when compared with rats bearing empty capsules, but not when compared with untreated rats. This apparent difference is most likely a result of relatively small numbers and large variability.

Bromocriptine was less potent than tamoxifen in blocking the estradiol-induced increases in pituitary wet weight and serum prolactin concentrations. Time-dependent increases in both parameters indicate that bromocriptine is less effective than tamoxifen in long-term treatment. If affected tissues become less responsive to bromocriptine with increasing treatment period, a higher dose of bromocriptine might be more effective at the later time points. A second factor influencing the effectiveness of bromocriptine in the model is its short half-life relative to tamoxifen. Because the estradiol was present continuously, it is possible that multiple injections of bromocriptine would be more effective.

The ability of either antiestrogens or dopaminergic compounds to stabilize or to reverse the effects of estradiol stimulation on pituitary wet weights and serum prolactin levels has not been studied in any animal model, although bromocriptine is widely used clinically to treat hyperprolactinemia and to reduce the size of prolactin-secreting pituitary tumors prior to surgery.[1,8,9] If it is assumed that the serum prolactin concentrations and pituitary wet weights of the delayed treatment groups at 8 weeks were not different from those of animals treated with estradiol alone and killed at 8 weeks, several conclusions can be drawn. Delayed treatment with this dose of tamoxifen arrested further pituitary growth due to the estradiol stimulus, but did not reduce pituitary wet weights to values seen at any time point in control animals or in animals treated throughout the experiment with tamoxifen. Delayed treatment with this dose of bromocriptine resulted in a small but significant decrease in pituitary wet weights, although pituitary weights were still significantly greater than those seen either in control animals or in animals treated for 12 weeks with bromocriptine. Following delayed treatment with either drug, serum prolactin concentrations were not significantly different from those in rats treated with estradiol alone for 8 weeks, and were significantly greater than either those of control animals or of animals treated for 12 weeks with estradiol and either drug. The more potent effect of bromocriptine in the delayed treatment groups and its less potent effect when given for 12 weeks suggest that the pituitary may become refractory to bromocriptine after extended treatment. This would also account in part for the greater effectiveness of tamoxifen with long-term treatment.

It is of interest that the animals bearing empty capsules and receiving vehicle injections had serum prolactin concentrations which were significantly greater than those of untreated controls, and that this increase was time dependent. This finding suggests that the stress associated with these experimental manipulations has a significant effect on prolactin secretion. However, this effect is small when compared with the effect of estradiol stimulation.

In summary, we have presented a useful model in which hypotheses concerning the mechanisms of action of estradiol in the induction of mammotroph hypertrophy and hyperplasia can be tested; we have found that, at the doses used, simultaneous treatment with tamoxifen is totally effective in blocking estradiol-induced pituitary weight gain and hyperprolactinemia, whereas treatment with bromocriptine is less effective; and we have found that delayed treatment with tamoxifen at this dose in the continued presence of estradiol stabilized but did not reverse the effects of estrogens, whereas treatment with bromocriptine reduced pituitary wet weight.

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