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Reduction of Serum Prolactin in Rats by 2 Ergot Alkaloids and 2 Ergoline Derivatives: A Comparison[1] (38207)

CHARLES L. BROOKS AND CLIFFORD W. WELSCH[2]
Department of Anatomy, Michigan State University, East Lansing, Michigan 48824

Suppression of the secretion of pituitary prolactin by the use of drugs (1–4) has been possible for only a few years, although increasing the secretion of this hormone by drugs has been feasible for many years (5). Ergocornine[3] (Ergo) and/or 2-bromo-α-ergocryptine (CB-154), 2 chemically similar natural alkaloids extracted from ergot, have been reported to terminate early pregnancy (6), prevent deciduoma formation (7), suppress mammogenesis (4) and inhibit lactation and luteolysis (8) in rodents, all of which imply suppression of prolactin secretion. More recently, Ergo when administered to rats (1–4) and CB-154 when administered to cows (9) or humans (10) markedly reduced serum prolactin, as determined by radioimmunoassay, thus confirming and extending these earlier observations. Evidence indicates that the effects of these drugs are exerted at both the hypothalamic (1) and the pituitary (2, 3) levels.

Considerable attention has recently been given to a number of ergoline derivatives which also appear to suppress prolactin secretion (11). 6-Methyl-8-β-ergoline-acetonitrile (MEA) has been reported to interfere with pseudopregnancy (12), pregnancy (12) and mammogenesis (13) in mice and to prevent pregnancy (14) in rats. Recent evidence has also been provided showing that a chlorinated derivative of MEA, 2-chloro-6-methyl-8-β-ergoline-acetonitrile (Cl-MEA), is also capable of suppressing the secretion of this hormone (11).

The considerable interest in the prolactin suppressing abilities of these drugs (Ergo, CB-154, MEA, Cl-MEA) is due, at least in part, to the demonstrated importance of this hormone in murine mammary tumor development and growth (15, 16) and perhaps development and growth of human metastatic breast cancer (17–19). The growth rates of the Huggins carcinogen-induced rat mammary tumors and spontaneous rat mammary tumors have been shown to be highly prolactin sensitive as these tumors regress when the hosts are treated with either Ergo or CB-154 (4, 20, 21). Other studies have provided evidence that these ergots are effective in the prophylaxis of murine mammary tumorigenesis (22, 23). Recently, Schulz (24) reported that treatment with CB-154 induced an objective remission in human metastatic breast cancer. CB-154, Cl-MEA and MEA are currently being investigated intensively in this country for their potential use in humans. In Europe and in Canada CB-154 has already been evaluated in human subjects as a suppressor of prolactin secretion (10). However, considerable additional knowledge needs to be acquired regarding the pharmacological acceptability of these drugs in man and in domestic and laboratory animals prior to extensive clinical use.

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The relative effectiveness of the natural ergot alkaloids (Ergo, CB-154) and the ergoline derivatives (MEA, Cl-MEA) as inhibitors of prolactin secretion has not been thoroughly investigated. The purpose of the present study is: (1) to measure immunoreactive serum prolactin levels in rats treated with Ergo, CB-154, MEA and Cl-MEA, and (2) to compare the 4 alkaloids with regard to the intensity and duration of their effects, in normal male and estrogen-stimulated female rats.

Materials and Methods. I. *Effect of ergo, CB-154, MEA and Cl-MEA on serum prolactin levels in male rats.* (a) Sixty, 2-month-old male Sprague–Dawley rats (Spartan Research Animals, Inc., Haslett, MI) weighing 270–350 g were randomly divided into 5 groups. They were housed in a temperature-controlled (75° ± 1°F) and light-controlled (14 hr light/day) room, fed a standard rat chow (Allied Mills, Chicago, Ill.) and water *ad libitum*. The groups and their treatments were: group I, controls; group II, Ergo; group III, CB-154; group IV, MEA and group V, Cl-MEA.

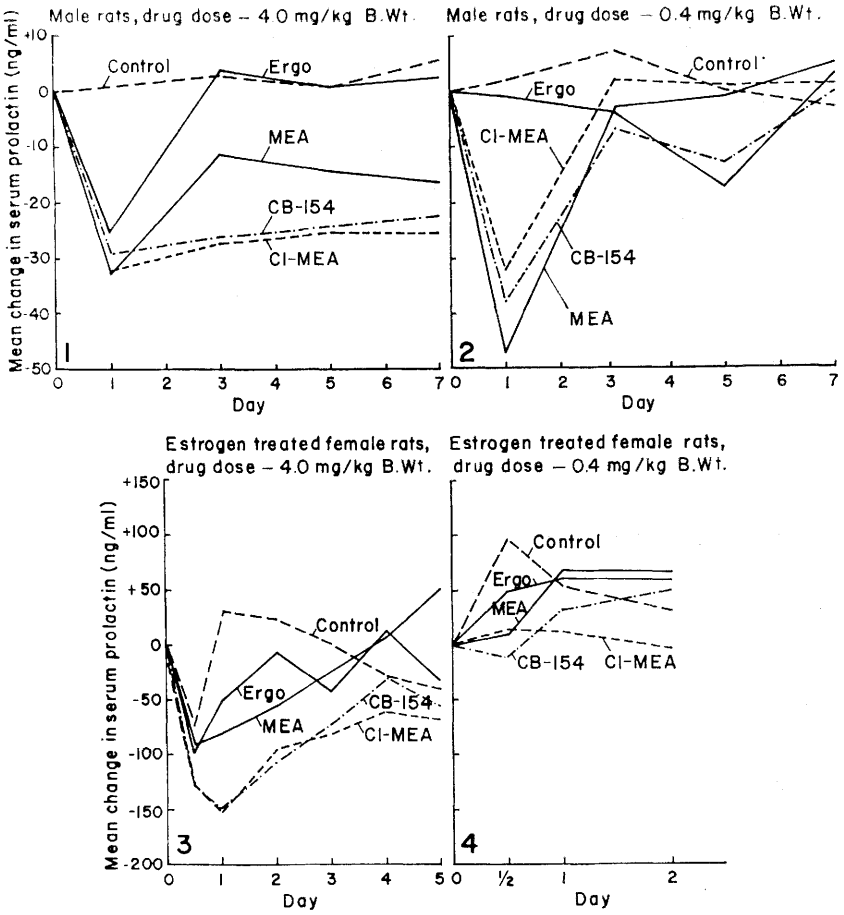
At mid-day on day 0, all animals were placed under light ether anesthesia for 2 min. One ml of blood was then withdrawn from each animal by cardiac puncture and immediately thereafter each animal was given a single sc injection of the appropriate drug at a dose of 4.0 mg/kg body wt. Controls received the vehicle only. Additional blood samples were obtained using the same method at days 1, 3, 5 and 7. The serum was analyzed for prolactin by double antibody radioimmunoassay[4] using a modification of the procedure of Niswender *et al.* (25). Statistical analysis was performed by calculating the mean increase or decrease of serum prolactin levels (ng/ml) for each group and comparing the means of days 1, 3, 5 and 7 to the mean of day 0. The significance of difference between means was calculated by the Student's *t* test. *P* values equal to or less than 0.05 were considered significant. b. Fifty-five 2-month-old male rats were divided into 5 groups and treated as described in (Ia) except that the animals received a lower dose of the drug (0.4 mg/kg body wt).

II. *Effect of ergo, CB-154, MEA, and Cl-MEA on serum prolactin levels in estrogen-treated female rats.* a. Sixty 2-month-old female Sprague–Dawley rats weighing 170–230 g were grouped as described in (Ia). For 5 days prior to and for 4 days following the administration of the drugs each animal received a daily sc injection of 10 μg of estradiol benzoate dissolved in 0.5 ml corn oil. On day 0, one ml of blood was obtained from each rat by cardiac puncture and immediately thereafter each animal was given a single sc injection of the appropriate drug at a dose of 4.0 mg/kg body wt. Additional blood samples were obtained at ½, 1–5 days after treatment with the drug. Blood was processed and prolactin analyzed as described in (Ia). b. Forty-three 2-month-old female rats were divided into 5 groups and treated as described in (IIa), except the animals received a lower dose of the drug (0.4 mg/kg body wt.) and blood was drawn only at 0, ½, 1 and 2 days.

The drugs were prepared by dissolving the mesylate salts in a minimal amount of 100% ethanol and diluting with 0.9% saline yielding a final concentration of either 1.0 mg/ml (high dose) or 0.1 mg/ml (low dose) of the drug. Final ethanol concentrations did not exceed 1%.

Results. A single injection of 4.0 mg/kg body wt. of either CB-154, MEA or Cl-MEA in male rats resulted in a significant reduction in serum prolactin levels for up to 7 days after treatment when compared to controls (Fig. 1). Ergo at the same dose level caused a significant reduction in serum prolactin at day one only. At the lower dose level (0.4 mg/kg body wt) CB-154, MEA and Cl-MEA significantly reduced serum prolactin levels only at day one (Fig. 2). Ergo was without significant effect at this dose level. No significant differences between CB-154, MEA and Cl-MEA in

[4] The Rat Prolactin Radioimmunoassay Kit was supplied by Dr. Albert Parlow through the National Institute of Arthritis and Metabolic Disease, Rat Pituitary Hormone Program, National Institute of Health.



FIGS. 1–4. Reduction of serum prolactin levels by 2 natural ergots and 2 ergoline derivatives. Ergo (Ergocornine); CB-154 (2-bromo-α-ergocryptine); MEA (6-methyl-8-β-ergoline-acetonitrile); Cl-MEA (d-2-chloro-6-methyl-8-β-ergoline-acetonitrile). Mean serum prolactin levels ± standard error for day 0 are (ng/ml): Figure 1. Control, 62.7 ± 5.1; Ergo, 35.6 ± 3.9; CB-154, 35.0 ± 5.7; MEA, 38.4 ± 7.2; Cl-MEA, 37.0 ± 4.2. Figure 2. Control, 58.3 ± 5.3; Ergo 57.9 ± 4.8; CB-154, 54.3 ± 6.8; MEA, 54.6 ± 5.8; Cl-MEA, 49.3 ± 6.3. Figure 3. Control, 157.6 ± 34.2; Ergo, 151.7 ± 28.1; CB-154, 178.4 ± 9.5; MEA, 147.0 ± 25.9; Cl-MEA, 130.3 ± 24.7. Figure 4. Control, 129.3 ± 20.8; Ergo, 130.5 ± 14.9; CB-154, 147.3 ± 35.0; MEA, 121.2 ± 18.6; Cl-MEA, 201.8 ± 34.4.

the intensity or duration of the reduction of serum prolactin were observed in these animals.

A single injection of 4.0 mg/kg body wt of any one of the 4 drugs to estrogen-treated female rats resulted in a significant reduction in serum prolactin levels one day after injection in contrast to the controls (Fig. 3). CB-154 and Cl-MEA-treated rats still had significantly reduced serum levels of prolactin 3 days after treatment. At the lower dose (0.4 mg/kg body wt), CB-154, MEA and Cl-MEA caused a significantly reduced level of serum prolactin only at 12 hr after injection (Fig. 4). Ergo at this lower dose level was ineffective in influencing serum prolactin levels.

Discussion. Four alkaloids, 2 of which were extracted from ergot (Ergo and CB-154) and 2 of which were synthesized from lysergic acid (MEA and Cl-MEA), were evaluated with regard to the intensity and duration of prolactin suppression, at 2 different dose levels, in male and estrogen-

treated female rats. CB-154 and Cl-MEA, followed closely by MEA, were found to be the most effective in reducing serum levels of prolactin, while Ergo was the least effective. Furthermore, a single injection of either CB-154 or Cl-MEA (4.0 mg/kg body wt) caused a significantly reduced level of serum prolactin for up to 7 days in male rats and up to 3 days in estrogen-treated female rats.

There have been a number of reports demonstrating that the administration of Ergo to rats results in a reduction of serum prolactin, as analyzed by radioimmunoassay (1–4). Indirect evidence in rodents, e.g., inhibition of mammogenesis, fecundity and lactation (26), has suggested that CB-154 also is an effective suppressor of prolactin secretion, although the direct measurement of this hormone by radioimmunoassay in rats has not as yet been reported. The results of this study demonstrate that CB-154 is capable of markedly reducing serum prolactin in rats and is distinctly superior to Ergo in its effect. Since CB-154 is also notably less toxic than Ergo (26), CB-154 appears to be pharmacologically preferable to Ergo.

Evidence has also implicated MEA as an inhibitor of prolactin secretion, as this drug has been reported to inhibit mammogenesis (13), mammary tumorigenesis (22), pseudopregnancy (12) and pregnancy (14) in rodents. The results of this study have shown that this drug, as well as the chlorinated derivative (Cl-MEA), sharply reduced serum prolactin in rats to a degree comparable to CB-154. Only when high doses of the drugs were administered to estrogen-treated female rats was MEA not quite as effective as CB-154. In accord, we have reported that MEA is an effective suppressor of normal and neoplastic mammary gland development in mice, but its effectiveness is slightly less than that of CB-154 (22). However, mice appear to tolerate MEA better than CB-154. The ergoline derivatives appear to lack the vasoconstrictive and emetic activities often associated with the ergot alkaloids (26). This suggests that the ergoline derivatives may be pharmacologically superior to the ergot alkaloids due to their relatively similar prolactin-suppressing abilities and less toxic effects.

Administration of estrogen to rats markedly increases the secretion of prolactin (27). The elevated prolactin levels of the estrogen-treated female rats, as reported in this study, are consistent with these earlier reports. It is apparent that these alkaloids, particularly CB-154, MEA and Cl-MEA, are effective in reducing not only normal secretory rates of the hormone, but also enhanced secretory rates such as that in estrogen-treated animals. Ergo has been previously reported to reduce serum levels of prolactin in rats treated with reserpine (28) and estrogen (2) or in rats bearing hypothalamic lesions (3), conditions in which prolactin secretion is increased. The results of the present study provide evidence that CB-154, MEA or Cl-MEA may be more effective than Ergo in curtailing hypersecretion of prolactin.

The ability to suppress prolactin secretion in humans can be of great value. Nonpuerperal galactorrhea has already been successfully controlled in humans by CB-154 (29). The recent interest in prolactin as a potentially important hormone in human breast tumorigenesis (17–19) as it is known to be in lower animals (13, 22, 28, 30) provides further impetus to acquire the means to effectively control the secretion of this hormone. The results of this study provide evidence that two ergoline derivatives (MEA and Cl-MEA), in addition to CB-154, may effectively provide these means.

Summary. Two purified natural ergot extracts (Ergocornine and CB-154) and 2 ergoline derivatives (MEA and Cl-MEA) were tested for the intensity and duration of prolactin-suppressing ability in Sprague–Dawley male rats and estrogen-treated female rats at two dose levels (4.0 and 0.4 mg/kg body wt). CB-154 and Cl-MEA followed closely by MEA were found to be the most effective, ergocornine the least. A single injection of the high dose of CB-154 or Cl-MEA significantly reduced serum prolactin levels in male rats for up to 7 days, and in estrogen-treated female rats for up to 3 days.

At the lower dose level, a single injection

of CB-154, Cl-MEA or MEA significantly reduced serum prolactin for up to 3 days in male rats and up to 12 hr in estrogen-treated female rats. Thus, data regarding the comparative effectiveness of prolactin suppressing drugs, i.e., 2 commonly used natural ergot alkaloids and 2 relatively new synthetic ergoline derivatives, have been provided in this study.

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