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Serum Prolactin Levels of Rats Under Continuous Estrogen Stimulation and 2 Br-α-Ergocryptine (CB-154) Injection[1] (38800)

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The regulation of prolactin secretion has been the subject of intense study in recent years, spearheaded by the advent of sensitive and specific radioimmunoassays (1, 2). As a result of these studies, numerous drugs have been discovered which either increase or decrease the secretion of prolactin *in vivo* (3). One compound, 2 Br-α-ergocryptine (CB-154), derived from the ergot class of drugs, has been demonstrated to specifically inhibit prolactin both *in vivo* and *in vitro* (4). Evidence at present indicates that it probably acts directly on lactotroph cells of the anterior pituitary preventing the release of prolactin (5, 6). There is some evidence that when prolactin release is blocked by ergot drugs there is an acute increase in pituitary prolactin content (5, 7). The purpose of this study was to determine if continuous CB-154 administration could block the influence of chronic estrogen stimulation on prolactin release.

Materials and Methods. A total of 50 female Sprague–Dawley rats 200–225 g in body weight were used in this study. The animals were divided into five groups of 10 animals each: Group I, sham controls, intact animals receiving 0.2 ml/day of a 0.5% tartaric acid solution; Group II, ovariectomized animals receiving 0.2 ml/day of a 0.5% tartaric acid solution; Group III, ovariectomized animals that were injected once every 21 days with 1 mg of polyestradiol phosphate (Estradurin) and daily with tartaric acid; Group IV, intact animals receiving 1 mg/day of 2 Br-α-ergocryptine (CB-154) in a 0.5% tartaric acid solution; Group V, ovariectomized animals receiving a combination of 1 mg/day of CB-154 and 1 mg of polyestradiol phosphate every 21 days. All animals were given the injections subcutaneously. Ovariectomies were performed 5 days prior to injections. A single injection of 1 mg of polyestradiol phosphate maintains vaginal estrus in ovariectomized rats for 3–4 wk (Lawson and Gala, unpublished observations). All animals were weighed weekly and vaginal smears were obtained from estrogenized animals to verify that they were under estrogen stimulation. Blood samples were obtained 6, 14, 21, 28, 35, and 42 days after the initiation of injection by orbital sinus puncture under ether anesthesia. Animals were under ether anesthesia for a standard 5-min period before the blood sample was obtained. At the end of 42 days, all animals were sacrificed and ovaries from sham control animals and intact animals injected with CB-154 were obtained and weighed. Serum samples were assayed for prolactin content at one dilution in triplicate using a specific rat prolactin radioimmunoassay as reported previously (8). The prolactin standard was NIAMDD rat prolactin RP-1. Differences between means were assessed statistically using Student's *t* test.

Results. The injection of 1 mg/day for 42 days of CB-154 did not alter body weight when compared to sham controls but did produce significantly larger ($P < 0.01$) ovaries (Table I). Ovariectomy significantly lowered serum prolactin when compared with sham controls at 6 days ($P < 0.01$), 14 days ($P < 0.01$), and 21 days ($P < 0.05$), but not at 28, 35, and 42 days (Fig. 1). Serum prolactin levels of ovariectomized rats at 6 and 14 days were significantly lower ($P < 0.05$) than those at 21, 28, 35, and 42 days. The serum prolactin levels of estrogenized animals were significantly higher ($P < 0.01$) after the second injection of 1 mg polyestradiol phosphate than after the first injection (28, 35, and 42 days vs 6, 14 and 21 days). Animals administered CB-154 alone had similar serum prolactin levels throughout each time period examined (Fig. 1). The combined administration of CB-154 and polyestradiol resulted in prolactin levels sig-

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TABLE I. INFLUENCE OF 1 MG/DAY OF CB-154 (2 BR-α-ERGOCRYPTINE) FOR 42 DAYS ON OVARIAN AND BODY WEIGHTS.

Group	Number animals	Body weight (gm)	Ovarian weight (mg)	
			Actual	/100 g BW
Sham controls	10	274 ± 6.0[a]	69.2 ± 4.6	25.3 ± 1.7
CB-154	10	262 ± 4.0[b]	192.8 ± 13.1	73.7 ± 5.0[c]

[a] Mean ± standard error.
[b] Not significantly different ($P > 0.05$) from sham controls.
[c] Significantly different ($P < 0.01$) from sham controls.

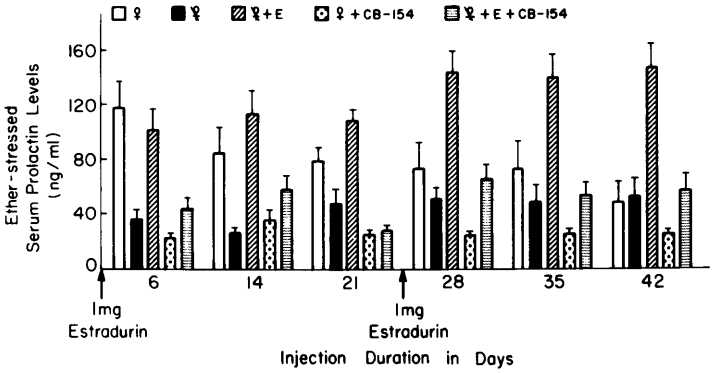

FIG. 1. The influence of polyestradiol phosphate (Estradurin) and 2 Br-α-ergocryptine alone or in combination on serum prolactin levels. Open bars represent values for intact sham control female rats receiving 0.2 ml/day of 0.5% tartaric acid solution. Closed bars represent values for ovariectomized animals receiving tartaric acid. Diagonally lined bars represent values for ovariectomized animals receiving 1 mg polyestradiol phosphate sc every 21 days. Dotted bars represent values for intact animals receiving 1 mg/day of CB-154 sc. Horizontally lined bars represent values for animals receiving both polyestradiol phosphate and CB-154. Vertical lines on the bars represent standard error of the mean. Statistically significant differences may be found in the text.

nificantly lower than those for polyestradiol alone ($P < 0.01$) but in most cases the values were higher than those of CB-154 alone: After the initial injection of polyestradiol, serum prolactin levels of animals given CB-154 were found significantly higher ($P < 0.05$) on the sixth day when compared with the respective values for animals receiving CB-154 alone. The difference was more apparent after the second injection of polyestradiol when prolactin values obtained on days 28, 35, and 42 were significantly different from CB-154 alone ($P < 0.01$, 0.05, and 0.05, respectively).

Discussion. The high prolactin values combined with an observed constant vaginal estrous smear indicates that animals injected with polyestradiol phosphate were under continuous estrogen stimulation for the duration of the experiment. The second injection of polyestradiol phosphate induced a slightly stronger stimulus to prolactin secretion and it was at this time that an override of the CB-154 block was most clearly observed. There have been reports that ergot drugs can block the release of prolactin but not its synthesis on the afternoon of proestrus (9, 10) and after suckling (11). Others have observed that an acute injection of estrogen to animals under ergot drug influence will increase pituitary prolactin content and/or synthesis, but the increase in pituitary size and the release of prolactin were blocked (5, 12). We have observed here that serum prolactin levels were significantly increased in estrogenized CB-154-blocked animals compared to animals receiving CB-154 alone (Fig. 1).

Our interpretation of the results is that

chronic estrogen can continue to stimulate prolactin synthesis despite the blockage of prolactin release which agrees with the observations of others after an acute estrogen stimulus. We further believe that ether anesthesia, if adequate, can act as a provocative stimulus to override the CB-154 block of prolactin release as evidenced by the increase in serum prolactin of the estrogenized-CB-154 animals. Valverde *et al.* (13) have observed that ether was capable of further increasing serum prolactin after the administration of reserpine and suggested that this anesthetic may be inducing the release of a prolactin-releasing substance. We have observed in other studies that ether provoked prolactin release in both ovariectomized and ovariectomized, estrogenized rats while other anesthetics provoked an increase in ovariectomized animals but a decrease in estrogenized animals (14, 15). Since TRH is the only known hypothalamic factor to date that stimulates prolactin release and it has little to no effect in ovariectomized rats (Lawson and Gala, unpublished), it does not appear that TRH is the hypothalamic factor suggested by Valverde *et al.* (13). One alternative to a specific prolactin hypothalamic releasing factor in mediating the action of ether may be a direct action of ether on the prolactin cells of the anterior pituitary to alter membrane permeability, thus inducing the release of stored hormone. Experiments are in progress to test this possibility.

Summary. The ability of 2 Br-α-ergocryptine (CB-154) to suppress serum prolactin levels was examined in animals under the influence of continuous estrogen stimulation. A single injection of polyestradiol phosphate (Estradurin) once every 21 days produced a constant elevation of serum prolactin. The simultaneous administration of 1 mg/day of CB-154 to estrogenized animals suppressed serum prolactin levels below that of Estradurin alone but the levels were significantly greater than those of animals receiving CB-154 alone. It was suggested that, while CB-154 prevents the release of prolactin, estrogen stimulates prolactin synthesis despite the block of its release. Ether anesthesia may be capable of partially overriding the block of CB-154 and released the stored hormone from the gland.

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1. Meites, J., and Clemens, J. A., Vitam. Horm. (New York) **30,** 165 (1972).
2. Frantz, A. G., *in* "Lactogenic Hormones, Fetal Nutrition and Lactation" (J. B. Josimovich, M. Reynolds, and E. Cobo, eds.), p. 379. Wiley, New York (1974).
3. Fluckiger, E., *in* "Prolactin and Carcinogenesis" (A. R. Boyns and K. Griffiths, eds.), p. 162. Alpha Omega Alpha Publ., Cardiff, Wales (1972).
4. Floss, H. G., Cassady, J. M., and Robbers, J. E., J. Pharm. Sci. **62,** 699 (1973).
5. Lu, K.-H., Koch, Y., and Meites, J., Endocrinology **89,** 229 (1971).
6. MacLeod, R. M., and Lehmeyer, J. E., Endocrinology **94,** 1077 (1974).
7. Kovacs, E., and Fluckiger, E., Experientia **30,** 1172 (1974).
8. Kuo, E. Y. H., and Gala, R. R., Biochim. Biophys. Acta **264,** 462 (1972).
9. Yokoyama, A., Tomogane, H., and Ota, K., Proc. Soc. Exp. Biol. Med. **140,** 169 (1972).
10. Yanai, R., and Nagasawa, H., Horm. Res. **5,** 1 (1974).
11. Fluckiger, E., and Kovacs, E., Experientia **30,** 1173 (1974).
12. Davies, C., Jacobi, J., Lloyd, H. H., and Meares, J. D., J. Endocrinol. **61,** 411 (1974).
13. Valverde, C., Chieffo, V., and Reichlin, S., Life Sci. **12,** 327 (1973).
14. Lawson, D. M., and Gala, R. R., J. Endocrinol. **62,** 75 (1974).
15. Lawson, D. M., and Gala, R. R., J. Endocrinol, in press (1975).

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