Effects of Estrogen Implant in Median Eminence on Serum and Pituitary Prolactin Levels in the Rat[1] (34323)

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Systemic estrogen treatment results in an increase in pituitary prolactin levels in the rat (1) and other species (2). Recently Chen and Meites (3) demonstrated that injections of several doses of estrogen in mature ovariectomized rats evoked an increase in serum as well as in pituitary prolactin levels. Prolactin release from the anterior pituitary *in vitro* (4) and serum prolactin (5) were much higher at estrus than during any other stage of the estrous cycle. Estrogen was shown to reduce hypothalamic prolactin inhibiting factor (PIF) content (6) and to directly stimulate the pituitary to release prolactin (7). Implantation of estrogen into the hypothalamus increased pituitary prolactin content, mammary gland development (8), and lactation (Yokoyama, personal communication). We recently found that estrogen implantation in the median eminence (ME) accelerates the growth of 7, 12-dimethylbenz(*a*) anthracene (DMBA)-induced mammary tumors in rats (manuscript in preparation). The serum and pituitary prolactin levels of these rats were investigated.

Materials and Methods. Mammary tumors were induced in female Sprague-Dawley rats, 55 days of age, by a single intravenous injection of DMBA.[3] At 15–30 days after the first palpable tumor appeared in each rat, or 45–80 days after DMBA administration, they were placed into the following groups: Ia, estradiol benzoate (EB) implant in ME; Ib, improper EB implant outside of ME; II, EB implant in the cerebral cortex; III, cholesterol implant in ME. An EB–cholesterol mixture at a ratio of 1:100, or cholesterol alone, were tamped into the end of a glass capillary tube of 23-gauge diameter, and implanted in the ME or in the cerebral cortex with the aid of a Stoelting stereotaxic instrument. This procedure was similar to that described by Clemens and Meites (9). The amount of EB in the glass tube was approximately 7–10 μg. Capillary tubes with a similar amount of EB were implanted subcutaneously into intact cycling rats to determine the systemic effects, if any, on prolactin secretion.

Daily vaginal smears were made in all animals throughout the experiment, beginning 10–15 days before implantation. At 25–28 days after implantation, when all rats in groups Ib, II, and III showed diestrous smears, they were killed by guillotine and blood was collected from the trunk and the serum was immediately frozen at —20°. The anterior pituitaries were removed and kept at —20°. All rats with an EB implant were examined for location of the implant. Rats in group Ia, all in diestrus, were killed 25 days after EB implantation. Rats with an improper EB implant, outside of the ME, were designated as group Ib. Serum and pituitary prolactin levels were measured by a recently developed radioimmunoassay for rat prolactin (10).

Results and Discussion. The estrous cycle was disrupted within a few days after EB implantation in the ME (Ia), and thereafter constant diestrus was observed. On the other hand, rats with EB implanted outside of the ME (Ib, II) or with a cholesterol implant in the ME (III), continued to show normal estrous cycles after implantation. Rats with

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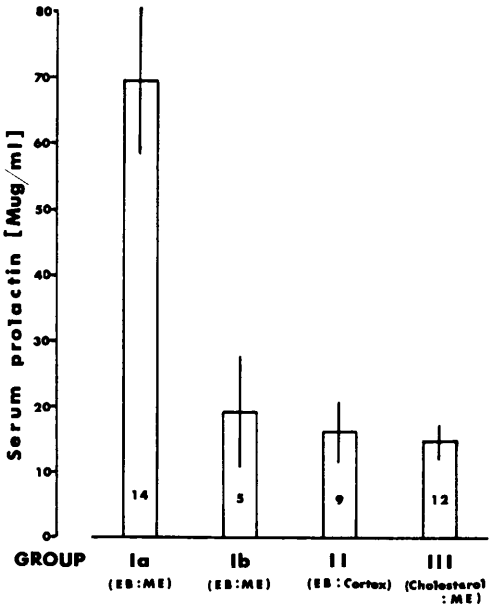

FIG. 1. Effects of EB or cholesterol implant in ME or cerebral cortex on serum prolactin levels of rats with mammary tumors; (center vertical bars), standard error of mean; number of rats is indicated at bottom of each column; Group Ib represents rats with improper EB implant outside of the ME.

the same amount of EB implanted subcutaneously also continued to cycle normally. Serum prolactin concentration in the rats with an EB implant in the ME (Ia) was significantly higher 25 days after implantation (Fig. 1) than in the other rats, and was comparable to levels in intact cycling rats during estrus (5). Serum prolactin concentration in the other rats (Ib, II, III) on the day of the first diestrus after 25 days of implantation was similar to untreated cycling rats in diestrus (5). Rats with an EB implant in the ME (Ia) had more pituitary prolactin content than the other rats, but there were no significant differences among rats in pituitary prolactin concentration (Fig. 2). Although mean pituitary weight of the former rats was somewhat higher than in the latter rats, this difference was not statistically significant.

The ratio of EB to cholesterol (1:100) employed for implantation in the present experiment was much smaller than used by other investigators. Ramirez and McCann (8) employed 1 part of estradiol to 2.5 or 5 parts of cholesterol to increase pituitary prolactin content and mammary gland development, and Yokoyama (personal communication) used 1 part of EB to 10 parts of cholesterol to promote lactation in rats. In a preliminary experiment, we implanted 1 part of EB with 5, 10, 100, 500, and 1000 parts of cholesterol, respectively, in the ME of mature ovariectomized rats, and found that a ratio of 1:5 or 1:1; evoked constant vaginal estrus in all animals whereas ratios of 1:100, 1:500, or 1:1000 did not alter the vaginal appearance of the ovariectomized rats. In almost all of the intact cycling rats implanted with EB in the ME at a ratio of 1:100 in the ME, the estrous cycles were disrupted within a few days after implantation and constant diestrus was observed for 20–35 days thereafter. On the other hand, implants of the same dose of EB elsewhere in the brain or subcutaneously had no effect on estrous cycles. Thus, the ratio of 1:100 employed in the present experiment eliminated any systemic action of the implanted EB, and its effects can be attributed entirely to an action on the hypothalamus, anterior pituitary or both.

These results indicate that the mammary tumors of the rats with an EB implant in the ME (Ia) were exposed to a higher level of prolactin than the rats under the other treat-

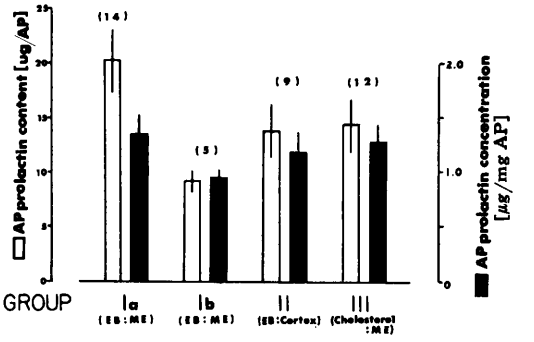

FIG. 2. Effects of EB or cholesterol implant in ME or cerebral cortex on anterior pituitary (AP) prolactin levels of mammary tumor-bearing rats. Vertical bars above represent standard error of mean. Number of animals is presented in parentheses. Group Ib represents rats with improper EB implant outside of the ME.

ments. This undoubtly accounts for the significantly greater growth of the mammary tumors observed in the former as compared to the latter rats (details will be published elsewhere).

Summary. Rats with DMBA-induced mammary tumors were given an implant of EB or cholesterol in the ME, or an implant of EB in the cerebral cortex or under the skin. At 25–28 days after implantation, serum prolactin levels in rats with an EB implant in the ME were significantly higher than in the other groups. There were no significant differences among groups in pituitary prolactin concentration, but pituitary prolactin content in rats with an EB implant in the ME was significantly higher than in the other groups. Whereas an implant of EB in the ME resulted in continuous diestrus within a few days, the same dose of EB implanted elsewhere did not disrupt regular estrous cycles. These results indicate that an estrogen implant in the ME can promote pituitary prolactin secretion by direct stimulation of the hypothalamus, pituitary, or both.

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