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ESTRADIOL IMPLANTS IN THE ARCUATE NUCLEUS INDUCE LACTOGENESIS IN VIRGIN RATS. ROLE OF PROGESTERONE.

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Summary

The aim of this study was to determine the effect of the centrally administered estradiol, and the effects of the consequent hypersecretion of prolactin (PRL) and progesterone, on lactogenesis as evaluated by mammary accumulation of casein and lactose. Bilateral cannulae containing 17β-estradiol or cholesterol were implanted in the arcuate nucleus of virgin rats on the day of estrus (Day 0). In the first experiment different groups of rats were killed on Days 6, 9, 15, 17, or 19. Trunk blood was collected and abdominal mammary glands were taken. In the second experiment, estradiol-implanted rats received the progesterone antagonist mifepristone or vehicle at 14.00 h on Day 8 or 16 post-implant, and were killed 28 or 48 h later. Serum PRL and progesterone and mammary casein were measured by RIA and lactose was determined by an enzymatic assay. Estradiol-implanted rats showed a significant increase in both milk components at all time points after implant compared to controls. On Day 9 after estradiol implant, mifepristone had no effect on mammary content of casein or lactose. By contrast, on Day 16, mifepristone markedly increased both casein and lactose contents without modifying serum PRL and progesterone concentrations.

In conclusion, 17β-estradiol implants in the arcuate nucleus of virgin rats results in hyperprolactinaemia and stimulates mammary accumulation of casein and lactose in the absence of feto-placental units. Despite the prolonged luteal activation, the sustained high levels of circulating progesterone become inhibitory to lactogenesis after a relatively long period after implant.

Key Words: mammary gland, mifepristone, hyperprolactinemia, prolactin, progesterone

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Estrogens stimulate the synthesis and the release of prolactin (PRL) in the rats and in other species by acting at both the adenohypophysis and hypothalamus (1). The arcuate nucleus appears to be the most important hypothalamic site mediating the estrogen action on the endocrine regulation of the adenohypophysis (2). Neurones expressing estrogen receptors are especially abundant in this nucleus (3). The implant of small cannulae containing estradiol into the arcuate nucleus of female rats provokes hypertrophy of lactotropes (4) and increases PRL release with a diurnal rhythm in the first three days after the implant, followed by a continuous hyperprolactinaemia and sustained high circulating progesterone levels (5).

Lactogenesis in rats, understood here as the increased accumulation of milk compounds that normally occur at the end of pregnancy, depends on hormonal regulation. The minimal hormonal requirements for the initiation of lactogenesis were early demonstrated to be PRL and corticoids (6). On the other hand, progesterone has been shown to inhibit lactogenesis in pregnant rats in vivo (7-12) and in vitro (13-15). Lactogenesis has most frequently been evaluated by the determination of mammary levels of β-casein and lactose (10-12).

The aim of the present study was to determine whether hyperprolactinaemia evoked by hypothalamic implants of estradiol is capable to induce lactogenesis in virgin rats, and if progesterone is an inhibitory factor in this model.

Materials and Methods

Animals

Virgin female rats, 3-4 months old (200-220 g) bred in our laboratory and originally of the Wistar strain were used. The animals were kept in a light- (lights on 06.00-20.00 h) and temperature-controlled room; rat chow (Nutric, Córdoba, Argentina) and tap water were available ad libitum.

Surgical procedures

All the animals were shown by vaginal smears to have had at least three successive four-day cycles. Bilateral implants were made stereotaxically under ether anaesthesia between 08.00 and 11.00 h on the estrous day. This day was designated as Day 0. Two groups were implanted; one, with steel cannulae (28 gauge, Small Parts, Miami, USA) containing 17β-estradiol (Roussel-Uclaf, Romainville, France) and the other one with cannulae containing cholesterol (Sigma Chemical Co., St. Louis, MO, USA), as a control group. Vaginal smears were taken daily after operation and estrogen-implanted rats showing a continuous diestrous vaginal smear were used. Cholesterol-implanted rats continued to have regular cycles and were used only on the day of diestrus for comparison with the estradiol group. All work was in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH publication N° 86-23, 1985).

In the first experiment different groups of rats were killed by decapitation on Days 6, 9, 15, 17, or 19. Trunk blood was collected and abdominal mammary glands were removed rapidly after decapitation and store frozen until the homogenates were performed.

In the second experiment, estradiol-implanted rats received mifepristone or vehicle at 14.00 h on Day 8 or 16 post-implant. Mifepristone-treated animals were killed 28 or 48 h after the injection on Day 8 and 28 h after the injection on Day 16, that is at 18.00 h on Day 9, at 14.00 h on Day 10 or at 18.00 h on Day 17 post-implant, respectively. Vehicle treated rats were killed 28 h after oil injection, at 18.00 h on Days 9 or 17 post-implant. The progesterone antagonist mifepristone (17β-hydroxy-11β(4-dimethylamino-phenyl)17α-(prop-1-ynyl)estra-4,9-dien-3-one, RU-486, kindly provided by Roussel-Uclaf, Romainville, France) was dissolved in sunflower seed oil at 1 mg/ml and injected s.c. at a dose of 1 mg/kg.

The removed mammary glands were stored frozen (-70 °C) until preparation of homogenates and blood was allowed to clot at room temperature and serum was separated and stored frozen (-30 °C) until assayed for PRL and progesterone. To determine the position of the cannulae, the brains from the implanted rats were removed after decapitation and serial frozen transverse sections were cut at 100 μm. Only rats with both cannulae positioned in the arcuate nucleus were included in the results.

Casein and lactose determinations

Mammary casein and lactose were measured as previously described (10,12). Briefly, 200 mg of mammary tissue were cut into small pieces and homogenised in 2 ml 50 mM sodium phosphate buffer, 150 mM NaCl, NaN_3 0.1 %, 0.1 % Triton X-100, pH 7.6 with an Ultraturrax homogeniser. The homogenate was centrifuged at 600 g for 30 min and the supernatant used for β-casein determination by a homologous radioimmunoassay according to Edery et al. (16) as modified in our laboratory (17), and lactose concentration was assessed by the method of Kuhn and Lowenstein (18).

Hormone assays

PRL was measured by a double-antibody RIA using materials kindly provided by Dr. S. Raiti, NIADDK Rat Pituitary Hormone Distribution Program. PRL was radioiodinated using the chloramine-T method. Results are expressed in terms of the rat PRL RP-3 standard preparation. Assay sensitivity was 1 μg/l serum and inter- and intra-assay coefficients of variation were 8 and 3%, respectively.

Serum progesterone was measured using a RIA developed in our laboratory (9) with antiserum raised in rabbits against progesterone-11-bovine serum albumin conjugate. Assay sensitivity was less than 16 nmol/l serum and inter- and intra- assay coefficients of variation were less than 10%.

Statistics

Values are given as means ± S.E.M. of groups of 6 to 9 animals. Comparisons between means of two groups were made by Student's t test. One-way analysis of variance (ANOVA I) was used for multiple comparisons. When variances were not homogeneous, logarithmic transformation of data was applied. Differences were considered significant if the probability was 5% or less.

Results

Effect of hypothalamic implants of 17β-estradiol on mammary casein and lactose contents.

Cholesterol-implanted rats showed low mammary contents of casein and lactose from Days 6 to 19 post-implant. The animals implanted with estradiol showed significantly higher ($p<0.05$) values in both milk components at all time points after implant compared to cholesterol-implanted animals (Fig. 1).

Effect of mifepristone on mammary casein and lactose contents and serum PRL and progesterone.

Mifepristone given on Day 9 to estradiol-implanted rats did not modify either casein nor lactose mammary contents 28 or 48 h after treatment compared to estradiol-implanted animals receiving vehicle (Fig. 2A). By contrast, mifepristone given on Day 16 to estradiol-implanted rats dramatically increased both mammary casein and lactose 28 h later (Fig. 2B).

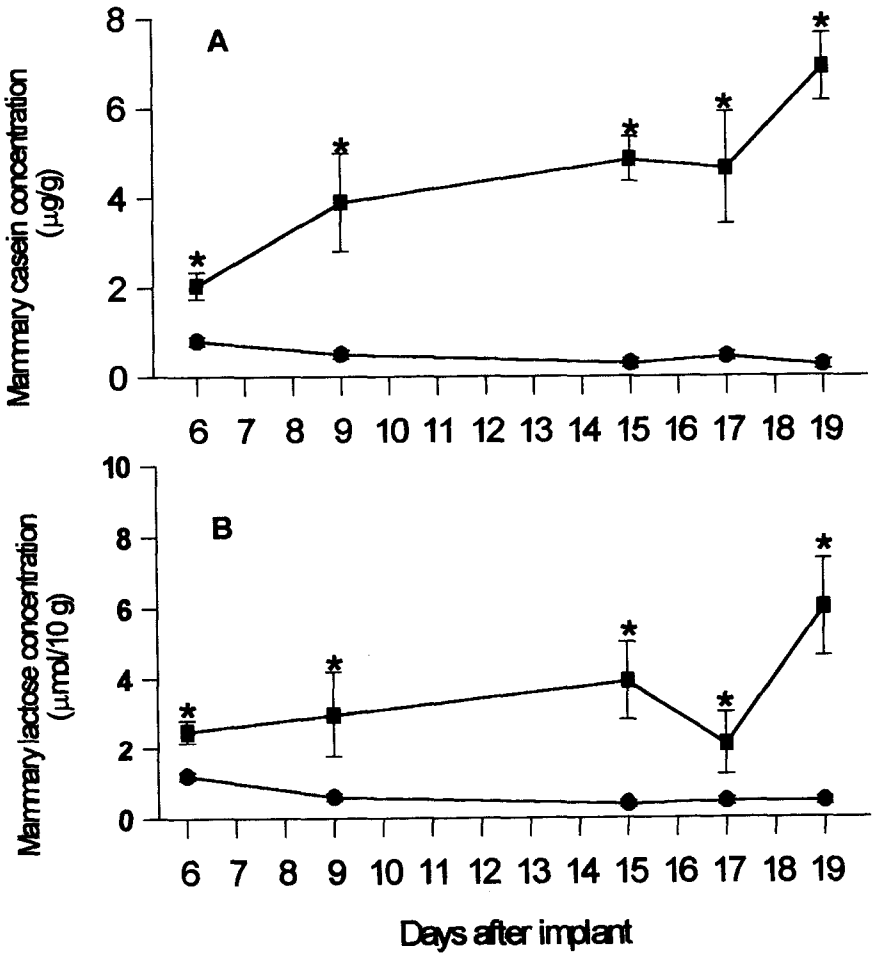

Fig. 1.

Mammary casein (A) and lactose (B) contents in virgin rats bearing implants of cholesterol (●) or 17β-estradiol (■) in the arcuate nucleus. Data are means ± SEM from 6-8 animals per group. Student's t-test was used for comparisons between groups for each time point. * $P < 0.05$ compared to the respective control group.

As in the first experiment, serum PRL and progesterone concentrations were significantly higher ($p<0.05$) at 18.00 h on Day 9 and at 14.00 h on Day 10 in the estrogen-implanted groups given vehicle versus cholesterol-implanted rats. In the estrogen-treated rats, serum PRL and progesterone values were not modified by treatment with mifepristone (Fig. 3). Serum PRL and progesterone concentrations were not significantly different at 18.00 h on Day 17 in the estrogen-implanted group treated with mifepristone compared to estradiol-implanted rats given vehicle, although a non-significant tendency to increase serum PRL and decrease circulating progesterone was observed (Fig. 3).

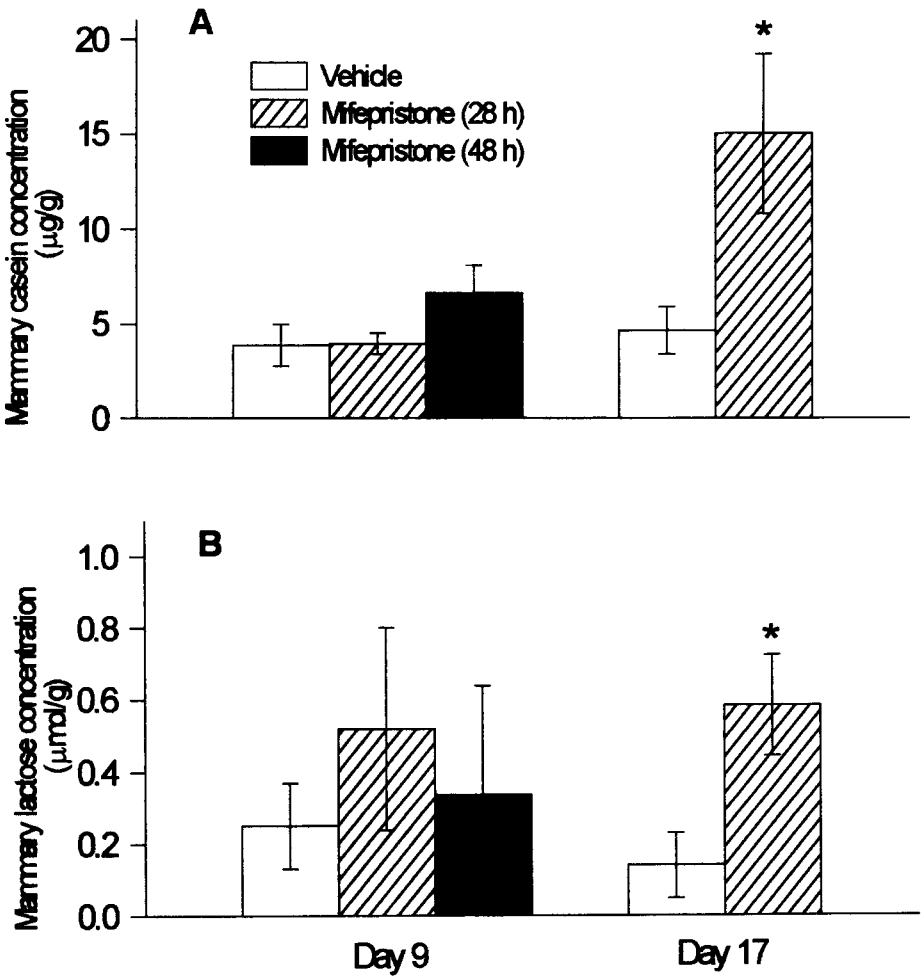

Fig. 2.

Mammary casein (A) and lactose (B) contents in virgin rats bearing implants of 17β-estradiol in the arcuate nucleus. Vehicle (open bars) or mifepristone (1 mg/kg, shaded bars) were administered 28 h before decapitation on Days 9 or 17. An additional group received mifepristone 48 h before decapitation on Day 9 (black bars). Data are means ± SEM from 6-8 animals per group. ANOVA-I and Student's t-test were used to compare values from Days 9 and 17, respectively . * $P < 0.05$ compared to the vehicle-treated group.

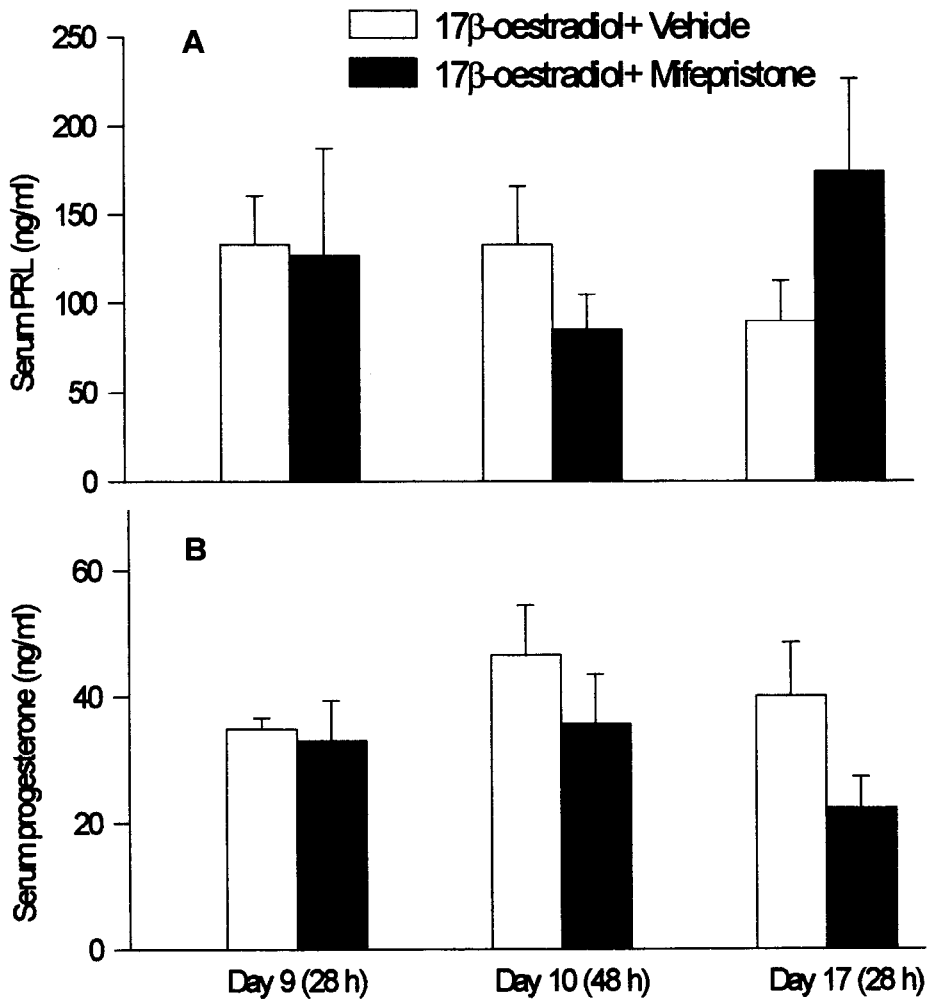

Fig. 3.

Serum concentrations of PRL (A) and progesterone (B) on Days 9, 10 and 17 post-implant, 28 or 48 h (indicated between brackets) after administration of vehicle or mifepristone. Rats received vehicle (open bars) or mifepristone (black bars, 1 mg/kg) at 14.00 h on Day 8 or 16 post- estradiol implant and were decapitated 28 h or 48 h later. Data are means ± SEM from 6-8 animals per group. Student t-tests were used for comparisons with the respective vehicle-treated group.

Discussion

Our results show that estradiol implants into the arcuate nucleus provoke an increase in mammary casein and lactose concentrations, evident about 6 days after implant. Furthermore, there is a clearly different response to mifepristone on Day 9 compared to that on Day 17, showing that progesterone became inhibitory of lactogenesis later in the period studied.

We have previously reported that estradiol directly acting in the arcuate nucleus is capable of provoking a striking increase in serum PRL about 24 h after the operation, with a diurnal rhythm in the first three days after the implant, followed by continuous hyperprolactinaemia (5). The present study demonstrates that this rise in PRL secretion can induce a secretory response at the mammary level, increasing casein and lactose contents in the absence of placental lactogen.

Since the early studies by Lyons (1958) a few studies in vivo have been conducted to show the minimal hormonal requirement for initiating lactogenesis in rats (7,9,12,19-21). From these studies it has been well established that PRL and placental lactogen have potent lactogenic actions in the presence of corticoid hormones and estrogen, while progesterone inhibits lactogenesis in pregnant rats. A considerable number of studies in vitro has been published showing results consistent with those of the in vivo studies (22-31).

Since, in our implanted rats, systemic blood 17β–estradiol was not augmented by the estradiol implants in the arcuate nucleus (5), hyperprolactinaemia is likely the main factor responsible for the increase in the secretory activity of the mammary gland. Placental lactogen is capable of inducing lactogenesis in the absence of PRL in late pregnant rats treated with the dopaminergic agonist bromocriptine, which blocks the secretion of PRL (12). On the other hand, ovario-hysterectomy, performed at the end of pregnancy in rats to abolish feto-placental secretions, induces lactogenesis most probably due to an increase in PRL secretion 24 h after surgery (19) since the increase in mammary casein and lactose is also blocked by bromocriptine administered at the time of the surgery (12,19). Thus, both lactogenic hormones are able to act independently to induce synthesis of casein and lactose, supporting our present results showing lactogenesis in hyperprolactinemic rats in the absence of feto-placental units. However, we found lower values of mammary lactose in our estradiol-implanted rats compared to pregnant rats (12) but higher levels of mammary casein, suggesting a differential effect of lactogenic hormones on these two components of milk.

Moreover, in estrogen-implanted rats progesterone levels reached values comparable to those of pregnancy, permitting us to speculate that the hormonal milieu in these rats is adequate to induce full mammary activity, even in the absence of feto-placental units, and raising the question of whether the progesterone levels are inhibitory to lactogenesis as occurs in pregnancy (8-10,12,25,28,32,33). We used the progesterone antagonist mifepristone at a relatively low dose (1 mg/kg) to test the inhibitory effect of progesterone on lactogenesis. At this dose mifepristone did not alter PRL secretion and also did not increase the production of casein and lactose on Day 9 in estradiol-implanted rats. This lack of response to the progesterone antagonist was observed 28 or 48 h after treatment. However, 17 days after estradiol implants, the mammary accumulation of casein and lactose was strikingly enhanced 28 h after mifepristone administration, showing a clear inhibitory effect of progesterone at this time. This effect of mifepristone at a low dose is not mediated by PRL, suggesting the importance of a direct inhibitory effect of progesterone on lactogenesis. In pregnant rats progesterone similarly inhibits lactogenesis only at the end of pregnancy, after a period of stimulatory action on mammary development (8,32,34,35). In rodents, both circulating progesterone and progesterone receptors are involved in the mammary epithelial growth and differentiation and the secretory development during pregnancy (36). However, progesterone receptors in mammary epithelial cells decrease by day 12 of pregnancy, probably in response to the increasing levels of circulating progesterone (37) and the increase in the sensitivity of the mammary gland to the inhibitory effects of progesterone are not explained by changes in the number of the receptors.

Mifepristone blocks the proliferation in the mammary epithelium of mice (38) suggesting that an increase in the differentiation might therefore occur producing a consequent augment in the rate of synthesis of milk components and lactogenesis. The antiglucocorticoid effect of mifepristone appears not to be involved at the dose used, since corticoids stimulate lactogenesis (21) and an inhibitory response to mifepristone was not observed in the present study.

In conclusion, 17β-estradiol implants in the arcuate nucleus of virgin rats induce hyperprolactinaemia and thereby stimulate mammary synthesis of casein and lactose in the absence of feto-placental units. Although the corpora lutea are rapidly activated, the sustained high levels of circulating progesterone become inhibitory to lactogenesis only after a relatively long period after implant.

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