

Occurrence and Secretion of Prolactin in Fetal Mouse Pituitaries¹ (35672)

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In view of the importance currently attached to the role of prolactin in amphibian larval growth and metamorphosis (1, 2) and in fish osmoregulation (3), it is pertinent to raise the possibility of related functions for prolactin in mammalian ontogeny. The production of prolactin by the mammalian fetal pituitary has yet to be demonstrated. Furthermore, if such hypophyseal activity be present, the nature of its hypothalamic control, if any, is also of interest. The present experiments were undertaken mainly to obtain information on the presence and secretion of fetal prolactin in the mouse, as well as to approach the problem of its possible hypothalamic control.

Materials and Methods. Disc electrophoresis. One-hundred, 51, and thirty-six 18-day fetal pituitaries were taken from BALB/cCrgl mice, 45 from BALB/cfC3H mice, and 26 from A/Crgl mice; these lots of pituitaries were homogenized in phosphate buffer (pH 7.2; ionic strength, 0.05) and subjected to 10% acrylamide-gel electrophoresis to determine presence of a prolactin band similar to that in the adult (4). Eleven adult female A/Crgl mice were injected daily for 3 days with 1 μ g of estradiol-17 β and 10 μ g of reserpine. The day after the last injection, pituitaries were taken and homogenized in a glass homogenizer. The homogenate was subdivided and applied to 12 columns of 10%

acrylamide-gel. Electrophoresis was conducted with 2.5 mA of current per column. After electrophoresis, one gel was stained in amido black 10B and destained electrophoretically, while the others were frozen until 3 major bands were discernible in the stained column. The other 11 gels were then cut into 7 segments in accordance with the occurrence of bands in the stained gel. The 3 segments containing the 3 major bands were eluted by soaking overnight in Hanks' solution. Confirmation of the prolactin band from the adult was attained by pigeon-crop assay.

Organ culture. The technique of organ culture of mouse mammary tissues with mouse fetal pituitaries of different stages was described previously (5). Twelve-day-pregnant nulliparous A/Crgl mice provided mammary tissues which were cultured for 3 days in Waymouth's medium (MB 752/1), supplemented with insulin (5 μ g/ml) and aldosterone (1 μ g/ml), with 5 or 10 pituitaries from each stage, ranging from 13- to 18-day fetuses. Stage of pregnancy was estimated by observing the appearance of vaginal plugs, counting the day of plug occurrence as day zero. Cultured mammary explants were fixed in Tellyesniczky's formalin-acetic acid-alcohol, sectioned serially at 7 μ , and stained with hematoxylin and eosin. Responses of mammary glands, judged from coded slides as described earlier (5), were compared with tissues incubated with ovine prolactin (NIH-P-S8, 5 μ g/ml). In two experiments, 16- or 18-day fetal pituitaries were incubated with mammary glands in a hypothalamic homogenate to determine any influence on prolactin secretion. Fresh hypothalami were collected and homogenized with a glass homogenizer in Waymouth's medium supplemented with insulin

¹ This study was supported by NIH grant CA 05388. We are indebted to Dr. C. S. Nicoll and Mr. C. W. Nichols, Jr. for the pigeon-crop assays, and to Mr. W. Jackson for microtechnical assistance. Prolactin was a gift of the NIH Endocrinology Study Section.

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and aldosterone. The homogenate was then passed through a Millipore filter (0.45 μ). In one experiment, 10 entire hypothalamus-pituitary complexes taken from 17-day fetuses were incubated with mammary tissues to examine the possible operation of prolactin-inhibiting hypothalamic hypophysiotropin by diffusion *in vitro*.

Results. Diagrams of sample electrophoretograms of adult and fetal pituitaries are shown in Fig. 1. Three distinct major bands were seen when one adult pituitary was used. The fastest band showed crop-stimulating activity and is considered to be prolactin. The second band is identified as albumin from its mobility comparable to that of commercial mouse serum albumin. The third band is regarded as growth hormone from its mobility similar to that described by Cheever *et al.* (4). When 100 fetal pituitaries were tested, the prolactin band was seen clearly. When 51 or 45 pituitaries were used, the prolactin band was visible but faint. With 36

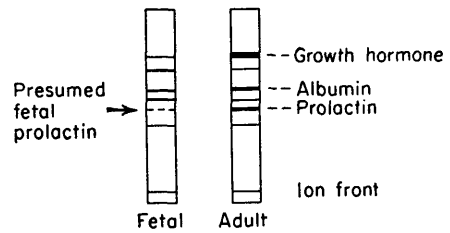


FIG. 1. Electrophoretogram of 18-day fetal and adult mouse pituitaries.

and 26 pituitaries, no prolactin band was visible. Thus, fetal pituitaries appear to contain small amounts of prolactin similar to adult prolactin.

The results of organ culture are summarized in Table I. Thirteen- and 14-day fetal pituitaries did not stimulate mammary secretion, whereas 15- to 18-day fetal pituitaries were effective. In any one experiment, mammary tissues cultured with 10 pituitaries always showed more secretion than those with 5 pituitaries. In three experiments, slides of

TABLE I. Response of Mouse Mammary Tissues Cultured with Fetal Mouse Pituitaries from Different Stages of Pregnancy.

| Age of fetuses (days) | No. of pituitaries | Secretory activity of mammary tissues | | |
|-----------------------|--------------------|---------------------------------------|-------------------|----------------|
| | | With pituitaries | Control cultures | |
| | | | Without prolactin | With prolactin |
| 13 | 10 | + | + | +++ |
| | 5 | ± | | |
| 13 | 10 | + to +++ | + | ++ |
| | 5 | + | | |
| 14 | 10 | + | + | ++ |
| | 5 | ± | | |
| 15 | 10 | ++ | + | + ^b |
| | 5 | + | | |
| 16 | 10 | ++ | + | + ^b |
| | 5 | ++ | | |
| 16 | 10 | ++ | + | ++ |
| 17 | 10 | ++ | + | ++ |
| 18 | 10 | ++ | ± to + | ++ |
| 18 | 10 | ++ | ± | ++ |
| | 5 | + to +++ | | |

^a ±, +, ++, +++ are grades of secretion in alveoli; ± indicating almost no secretion, +++ highly secretory.

^b Ovine prolactin was inexplicably ineffective in these control cultures.

mammary explants cultured with fetal pituitaries with and without homogenized or intact hypothalami were paired, coded, and arranged randomly before the amount of secretion was compared within each pair. Mammary tissues cultured with fetal pituitaries without hypothalamic homogenate always showed more secretion than those with fetal pituitaries with hypothalamic homogenate. No difference was detected in secretion of mammary tissues cultured with and without intact, connected hypothalami.

Discussion. Ceriani (6) reported that rat fetal mammary rudiments explanted in organ culture required prolactin in addition to insulin and aldosterone to achieve the same extent of ductal ramification as the mammary gland of the newborn. Insulin appears to be produced by the fetal pancreas, since placental transport of the maternal hormone seems unlikely (7). Maternal prolactin, of a larger molecular size than insulin, may also not cross the placenta. Kaplan and Grumbach (8) demonstrated that fetal human growth hormone and human placental lactogen do not cross the placenta. It is possible that any prolactin effective on the fetus would have to be of fetal pituitary origin.

Disc electrophoresis reveals the presence of prolactin in the fetal mouse pituitary on day 18 of pregnancy; co-culture indicates that there can be secretion of prolactin in the late stages of pregnancy. At earlier stages (13 and 14 days of gestation), the embryonic pituitary could still be secreting a small amount of prolactin; however, it was not detected with our organ-culture method.

Our preliminary experiments suggest that the fetal mouse hypothalamus at the end of gestation, may contain prolactin-inhibiting factor (PIF). That there is a dose-response relationship for mammary-secretory activity in the submaximal range of prolactin concentrations, is indicated by the fact that 10

pituitaries always resulted in greater stimulation than 5 pituitaries. Accordingly, the lower secretion of prolactin when pituitaries were cultured with hypothalamic homogenate, indicates the possible presence of fetal PIF.

In conclusion, it is evident that the fetal mouse pituitary contains prolactin and that it may secrete prolactin *in vitro* as early as 15 days of gestation. A prolactin-inhibiting factor may be present in the hypothalamus in late fetal life.

Summary. The presence of a prolactin band in 18-day fetal pituitaries was demonstrated by disc electrophoresis. Five or 10 pituitaries of 13- to 18-day fetal mice from A/Crgl strain were organ cultured with 12-day pregnant nulliparous mammary tissues of the same strain to investigate the capability of prolactin secretion by the fetal mouse pituitary. Mammary tissues cultured with pituitaries of 15- to 18-day fetuses showed secretory material in their alveoli, similar to that seen in cultures containing purified ovine prolactin. Late fetal pituitaries cultured in the presence of hypothalamic homogenate secreted less prolactin than in its absence, indicating the possibility of a prolactin-inhibiting factor in the fetus.

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Received Feb. 22, 1971. P.S.E.B.M., 1971, Vol. 137.