Termination of Pregnancy in Rats by a Prolactin Implant in Median Eminence* (33623)

J. A. CLEMENS,[1] M. SAR,[2] AND J. MEITES

Department of Physiology, Michigan State University, East Lansing, Michigan 48823

Recent experiments in our laboratory have demonstrated that a prolactin implant in the median eminence of rats increases hypothalamic PIF content, decreases pituitary prolactin concentration, induces mammary gland atrophy, inhibits postpartum milk secretion and results in regression of the corpora lutea of lactation (1, 2). It was also observed that prolactin implants in the median eminence of pseudopregnant rats resulted in early termination of pseudopregnancy and inhibition of deciduoma formation (3). It was of interest therefore, to determine the effects of a prolactin implant in the median eminence on maintenance of pregnancy in the rat.

Materials and Methods. Female adult Holtzman rats (Madison, Wisc.), 3 months old, were housed with males until pregnancy had occurred. Vaginal smears were made daily and the day spermatozoa were detected was designated as day 1 of pregnancy.

A single glass tube containing a prolactin (NIH-P-S7, 24 IU/mg)[3]–cocoa butter mixture was implanted stereotaxically into the median eminence of each of 6 rats in eight groups of pregnant rats by methods previously described (1). Each implant contained about 250 μg of prolactin. Eight other groups of pregnant rats (controls) received implants of glass tubes containing cocoa butter alone in the median eminence. The six rats from each group were implanted once on each day from day 1 until day 8 of pregnancy. Thus a total of 48 experimental rats received implants of the prolactin–cocoa butter mixture, and 48 control rats received cocoa butter alone.

TABLE I. Effects of a Prolactin Implant in Median Eminence on Maintenance of Pregnancy in Rats.

Type of implant (6 rats/group)	Day of pregnancy implant placed	No. of rats pregnant on day 15
Cocoa butter (controls)	1	6
	2	6
	3	6
	4	6
	5	6
	6	6
	7	6
	8	6
Prolactin–cocoa butter	1	0
	2	0
	3	0
	4	0
	5	0
	6	0
	7	5
	8	6

In another experiment 12 rats were divided equally into 2 groups on the fourth day of pregnancy. The first group served as controls, and each rat received an implant of cocoa butter and daily subcutaneous injections of 2 mg of progesterone from day 4 until day 11 of pregnancy. The second group of rats each received an implant of the prolactin–cocoa butter mixture on the fourth day of pegnancy and daily subcutaneous injections of progesterone from day 4 until day 11 of pregnancy. A laparotomy was performed on these rats on day 11 of pregnancy, and they were killed on day 15 of pregnancy.

Results. Table I shows that when prolactin–cocoa butter was implanted into the median eminence of pregnant rats from days 1–6, pregnancy was terminated in all cases. When

* Supported in part by NIH Research Grant AM-04784-08.

[1] NIH predoctoral trainee during tenure of this work (GM 1121-05). Present address: Department of Anatomy, University of California at Los Angeles, Los Angeles, California.

[2] Present address: Department of Pharmacology, University of Chicago, Chicago, Illinois.

[3] NIH-P-S7 prolactin was a gift from the Endocrinology Study Section, NIH.

TABLE II. Effects of a Prolactin Implant on Day 4 and Daily Progesterone Injections on Days 4–11 on Maintenance of Pregnancy.

Treatment	No. of rats pregnant on day 11	No. of rats pregnant on day 15, after withdrawal of progesterone on day 12
Cocoa butter implant + 2 mg progesterone daily	6	6
Prolactin–cocoa butter implant + 2 mg progesterone daily	6	0

laparotomies were performed on day 15, no fetuses were found in these rats. When prolactin–cocoa butter was implanted on day 7, 5 out of 6 rats remained pregnant; when the mixture was implanted on day 8, all 6 rats remained pregnant. Implants of cocoa butter alone had no effect on pregnancy, regardless of the day on which they were implanted. At laparotomy on day 15, both the number and size of fetuses appeared to be normal in these control rats.

Table II shows that when cocoa butter alone was implanted on the fourth day of pregnancy and 2 mg of progesterone were administered daily until day 11, pregnancy was maintained until the rats were killed on day 15. When prolactin–cocoa butter was implanted and 2 mg of progesterone were injected daily from days 4–11 of gestation, pregnancy was also maintained as determined by laparotomy on day 11. However, when progesterone treatment was withdrawn after day 11, resportion or abortion occurred in all of these rats.

Discussion. These results demonstrate that implantation of prolactin into the median eminence during the first 6 days of gestation results in termination of pregnancy in the rat. This suggests that adequate pituitary prolactin secretion is necessary for at least the first 6 days of gestation to maintain pregnancy in the rat. The probable decreased pituitary prolactin secretion in the prolactin-implanted rats after the sixth day had very little or no effect on the pregnant state. This is probably due to secretion of luteotropin by the newly formed placenta. Previously it had been reported that the pituitary was essential until midgestation in the rat, since hypophysectomy before this period usually resulted in resorption of fetuses (4). However, further work is necessary to establish whether this is due solely to loss of prolactin or to other causes as well.

The prolactin implant appeared to exert its effect by inducing luteal regression, resulting in a decrease in progesterone production. This conclusion is supported by the observation that daily administration of 2 mg of progesterone maintained pregnancy in rats given implants of prolactin. When the progesterone injections were discontinued after day 11 of pregnancy, the animals resorbed their young or aborted soon thereafter. In pseudopregnant (3) and postpartum rats (2), a prolactin implant in the median eminence also induced regression of the corpora lutea, providing further evidence that this is the mechanism by which the prolactin implant terminated pregnancy.

The present results indicate that injections of 2 mg of progesterone daily during days 4–11 of pregnancy to control rats, and withdrawal of progesterone thereafter, did not interfere with continuation of the pregnant state. On the other hand, once the corpora lutea had regressed in the prolactin-implanted rats, the corpora lutea could not be reactivated by placental luteotropin. Pregnancy was maintained in these rats only as long as progesterone was administered. These results provide additional evidence that prolactin can inhibit its own secretion by the pituitary, and thereby prevent its normal functions in the body.

Summary. A prolactin–cocoa butter mixture was implanted once in the median eminence of rats on each of days 1–8 of pregnancy. Control pregnant rats were given similar implants of cocoa butter alone. When laparo-

tomies were performed on day 15, all of the control rats were still pregnant. None of the rats implanted with prolactin–cocoa butter on days 1–6 remained pregnant, but almost all rats remained pregnant when implanted on days 7 or 8 of pregnancy. These results indicate that a prolactin implant in the median eminence, by reducing pituitary prolactin secretion, terminates pregnancy if given during the first 6 days of gestation. When prolactin was implanted in the median eminence of rats on the fourth day of gestation and 2 mg of progesterone were injected daily until day 11, pregnancy was maintained. When progesterone was withdrawn after day 11, and the rats were killed on day 15, it was evident that resorption of the fetuses had occurred. This indicates that the decrease in pituitary prolactin secretion produced by the implant of prolactin into the median eminence, resulted in luteal regression and reduced progesterone secretion.

1. Clemens, J. A. and Meites, J., Endocrinology 82, 878 (1968).

2. Clemens, J. A., Sar, M., and Meites, J., Federation Proc. 27, 319 (1968).

3. Chen, C. L., Voogt, J. L., and Meites, J., Endocrinology 83, 1273 (1968).

4. Zarrow, M. X., *in* "Sex and Internal Secretions", Vol. 2, (W. C. Young, ed.), Vol. 2, p. 1006. Williams and Wilkins, Baltimore, Maryland (1961).

Received Oct. 1, 1968. P.S.E.B.M., 1969, Vol. 130

Studies of Thyrocalcitonin Inactivation *in Vitro** (33624)

J. P. ALDRED AND ROBERT J. SCHLUETER

Department of Pharmacology and Biochemistry, Armour Pharmaceutical Company, Kankakee, Illinois 60960

Recent results of Tashjian and Voelkel (1) indicate the presence of a factor in a serum which inactivates thyrocalcitonin (TC) *in vitro*. Data to be presented below indicate the presence of another factor, or factors, in rat serum which prevents the rapid inactivation of partially purified TC occurring *in vitro* at neutral pH.

Methods. TC was prepared from pork thyroid glands by an acid extraction procedure (Schlueter and Fisher, unpublished). Most experiments were performed with one TC preparation (K322-176) containing 12 Medical Research Council (MRC) U/mg solids, although certain experiments utilized less pure material containing 0.12 U/mg (K322-096), or 0.03 U/mg (K412-045).

TC was incubated *in vitro* in siliconed glass tubes at a concentration of 60 MRC milliunits (mU) per ml in a medium of 0.1 *M* Tris buffer, pH 7.5, 0.1 *M* sodium acetate buffer, pH 5.5, or 0.9% NaCl at various pH levels. Incubation of TC was either for 30 min at 23°, or 4 hr at 37°.

After *in vitro* incubation, TC activity was bioassayed in 100–130-gm female Sprague-Dawley rats, fasted for 16 hr. Three rats were used per group. Subcutaneous injections of 0.5 ml/100 gm body weight were given, with blood sampling 1 hr post-treatment. Serum calcium was determined by EDTA titration (2). The data were analyzed using Student's *t* test.

Results. Inactivation of TC at Neutral pH. In contrast to the data of Tashjian and Voelkel (1) showing stability of TC activity at pH 7.5, preliminary experiments indicated a complete loss of hypocalcemic activity after *in vitro* incubation of TC (12 U/mg) for 4 hr at 37° in 0.1 *M* Tris buffer, pH 7.5. Likewise, hypocalcemic activity was lost when TC was incubated in pH 7.5 Tris buffer for 30 min at 23° (Table I), while TC, incubated in pH 3.2 saline, was not inactivated. Hypocalcemic activity was not recovered by acidifying the inactivated solution to pH 3.0 and incubation at 23° for 1 or 6 hr,

* This study was presented in part at the 52nd Annual Meeting of the Federation of American Societies for Experimental Biology, April 17, 1968.