PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 178, 531–535 (1985)

Failure of Injection of Rat Placental Lactogen to Inhibit Prolactin *in Vivo*[1] (42038)

JAMES L. VOOGT[2] AND ANNE SALAMON

Department of Physiology, University of Kansas Medical Center, Kansas City, Kansas 66103

Abstract. In an attempt to demonstrate a negative feedback of rat placental lactogen (rPL) on prolactin secretion, pregnant rats were hysterectomized and injected intraperitoneally with placental extracts. Hysterectomy alone prolonged the incidence of nocturnal prolactin surges and injection of placental extracts did not alter this response. However, the absence of rPL in the serum following the injections indicated a primary reason why no inhibition was seen. Only when rPL was given intravenously was there detectable amounts found in the blood. The slow disappearance of rPL from the circulation following hysterectomy in Day 11 pregnant rats suggests that the lack of rPL in the blood following ip injection of placental extracts is not due to rapid clearance of rPL from blood. The failure to show a negative feedback of rPL on prolactin *in vivo* may be due primarily to the lack of appearance of rPL in the circulation following an ip injection of placental extracts.

Numerous experiments have been done which suggest that the appearance and rapid increase in rat placental lactogen (rPL) secretion that occurs at midpregnancy terminate the twice daily surges of prolactin (PRL). Secretion of rPL and termination of PRL surges have been correlated with the number of conceptuses present (1, 2). A delay in the time of blastocysts implantation prolonged the number of days the PRL surges were present (3, 4) and delayed the appearance of rPL, proportionate to the length of implantation delay (4). However, all of these experiments only provide indirect or correlative evidence that rPL inhibits PRL. Several attempts to show that placental extracts given *in vivo* could inhibit PRL have failed (3, 5–7). In all cases these injections were given intraperitoneally, or subcutaneously, but at no time was the activity of rPL in the extracts or in the blood following injection ascertained. The purpose of the present study was to determine whether injection of placental extracts obtained from rats on Days 10 or 11 of pregnancy inhibited PRL surges in rats hysterectomized on Day 10, and resulted in measurable amounts of rPL in the peripheral circulation. Second, a measurement of rPL disappearance from the blood following hysterectomy on Day 11 or Day 17 was done to determine whether the magnitude of the half-life of rPL could help explain the lack of effectiveness of the placental extract injections to raise blood levels of rPL.

Material and Methods. *Animals.* Female Holtzman rats (Holtzman Co., Madison, Wis.) were housed in a temperature controlled room with lights on from 0600 to 1800 hr, and were given food and water *ad libitum.* For mating purposes three females were housed with one male and the morning sperm found in the vaginal lavage was designated Day 0. If rats were bled only once per day, blood was obtained via cardiac puncture, under light (less than 1 min) ether anesthesia. For those experiments in which multiple blood samples were taken in a short period of time, rats were cannulated via the right carotid artery using PE50 tubing. Blood (0.3 ml) was then withdrawn without using anesthesia or restraint, and an equal volume of saline returned to the rat.

Experiments. In the first experiment, all rats were hysterectomized on Day 10, between 0800 and 1000 hr. They were injected intraperitoneally with placental extracts at 1000 hr on Day 10, 0100 and 1000 hr on Day 11, and 0100 hr on Day 12. Rats were bled at 0500 hr on Days 10, 11, and 12. The placental extracts were prepared in 2% butyl alcohol: saline solution using the method of Matthies (8). The volume was adjusted so that each injection was the equivalent of five placentas.

[1] This work was supported in part by NIH Grant HD-15533.

[2] To whom reprint requests should be addressed.

0037-9727/85 $1.50

The first two injections were from Day 10 pregnant rats and the second two from Day 11 pregnant rats.

The second experiment was done to follow the appearance of rPL in the blood of recipient hysterectomized–ovariectomized rats following injection of either placental extracts, serum of pregnant rats, or media from placental incubations. In the initial group in this series, each rat was injected ip with the equivalent of six placentas from Day 11 pregnant rats and bled at 0, 2, 4, 6 and 24 hr. In the second group, the treatment was the same but blood samples were taken much more frequently (0, 10, 20, 30, 60, 90, 120 min) following injection. In the third group, rats were bled as in Group 2, but were injected ip with incubation medium containing rPL. This was obtained by incubating for 24 hr several flasks, each containing six placentas in 2 ml of medium 199. Following the incubation, the media were centrifuged for 30 min and filtered through a millipore filter. Media from several flasks were pooled and used for injection. The equivalent of rPL from six placentas was used for each injection. In the final two groups in this series, hysterectomized–ovariectomized rats were injected with either 1 ml serum from Day 11 or 12 pregnant rats, or media as described above. The major difference in these groups was that the injection was given via the carotid cannula, not intraperitoneally as was done in the previous groups.

The third experiment was done to determine the disappearance of rPL from the circulation following hysterectomy. In all cases, Day 11 or Day 17 pregnant rats were initially cannulated, followed by rapid hysterectomy or sham hysterectomy. In the first series, blood samples were taken at 0, 2, 4, 6, and 24 hr. In subsequent groups, blood sampling was done much more frequently in order to better characterize the disappearance curve of rPL. The times used were 0, 10, 20, 30, 60, 90, and 120 min following hysterectomy.

Hormone assays. Sera or plasma obtained by cardiac puncture or carotid cannula were kept at 4°C overnight, centrifuged, and stored at −70°C until assayed. Each sample was assayed in triplicate for prolactin using standard RIA methods. The assay materials were provided by the NIADDK Hormone Distribution Program, and the reference preparation was NIADDK rat PRL-RPI. The Nb_2 lymphoma cell bioassay for lactogenic hormones (9) was used, with our modifications (10), to measure rPL levels. This assay does not differentiate rPL I from rPL II. However, all measurements were done either before Day 13 when the major form in the plasma is rPL I or on Day 17, when only rPL II is present. Ovine PRL (NIH S-10) was used as the standard for the assay and all data are expressed as nanogram rPL/per milliliter. To neutralize the stimulatory effect prolactin present in the serum has on these lymphoma cells, antiserum to rat prolactin was added to each assay well during the assay. This antiserum (NIADDK anti-rPL-ICF-1), generously provided by Dr. Parlow, was used at a final concentration of 1:60,000 which was able to neutralize prolactin up to a concentration of 8000 ng/ml serum. Serum samples (0.2 or 1.2 μl) were assayed in duplicate and both the intra- and interassay coefficients of variation were 10%. Because each serum sample assayed used 1.2 μl as the maximum amount of serum, the limit of detection was about 25–50 ng/ml. Student's *t* test was used to compare means from experimental groups with the control group.

Results and Discussion. The effect of hysterectomy on Day 10 followed by twice daily injections of placental extracts on serum prolactin levels is found in Fig. 1. Control animals were injected with butanol:saline and hysterectomized or sham operated. As expected, all animals demonstrated high prolactin levels at 0500 hr on Day 10, which is during the nocturnal surge (5). Sham-operated rats lost the surge by Day 11, whereas hysterectomy extended the surges to Days 11 and 12. This agrees with an earlier report (6). Somewhat surprisingly, injection of placental extracts failed to inhibit these additional surges, even though the placentas used were from parallel stages of pregnancy. However, when one measured serum rPL, only the intact rats showed the presence of rPL on Days 11 and 12 (Fig. 2). Thus the injected rPL was either cleared from the blood in less than 4 hr or never diffused into the blood. Unfortunately, the amount of rPL in each injected extract was not determined. As noted

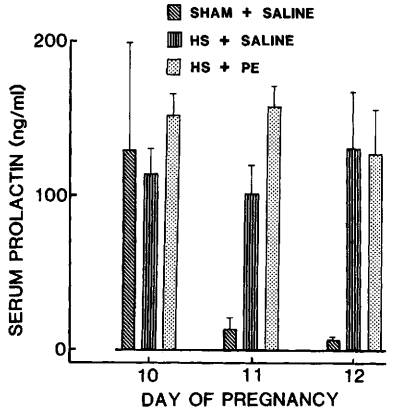

FIG. 1. Serum prolactin levels in hysterectomized (HS) rats injected with placental extract (PE). Hysterectomy was done on Day 10 of pregnancy and PE was injected at 1000 hr on Day 10, 0100 and 1000 hr on Day 11, and 0100 hr on Day 12. Rats were bled at 0500 hr. The HS groups had significantly higher ($P < 0.001$) prolactin levels on Day 11 and 12 than the sham group. The height of the bar represents the mean and vertical line the standard error of the mean in this and all subsequent figures.

later, this method for rPL extraction results in rPL concentrations ranging from 3000 to 4000 ng/injection. The inability of placental extracts to inhibit prolactin was described earlier by three laboratories. Smith and Neill (5) injected eight placental equivalents (Day 11) per day into pseudopregnant rats, and this did not inhibit the prolactin surges even though it was capable of maintaining corpora luteal function. Voogt (6) found that injections of two rat placentas from Day 12 pregnant rats at three different times did not inhibit the nocturnal prolactin surge in pregnant rats hysterectomized on Day 10. Yogev and Terkel (3) injected the equivalent of 10 placentas from Days 11 to 12 of pregnancy into lactating rats, but failed to alter the prolactin response to suckling. In the present experiment the lack of rPL in the blood precluded any effect rPL may have had on prolactin.

In order to more fully characterize blood levels of rPL following injection of solutions containing rPL, experiments were done in which blood samples were taken frequently following the injection. The amount of rPL injected was also determined. The first two groups of long-term hysterectomized rats were injected ip with Day 11 placental extracts. The equivalent of six placentas was injected into each rat, and the amount of rPL injected was 3147 ng per rat. In the group bled at 2-hr intervals, no rPL was detected in the blood at any time. A second group was bled at 10-min intervals after injection, and it also failed to have detectable levels of rPL in the circulation. When media from 24-hr incubation of Day 11 placentas were injected ip into long-term hysterectomized rats and blood samples were taken at 10-min intervals, no rPL was detected in the blood. The amount of rPL injected into each rat was 4096 ng. The number of rats used in each of the above ranged from five to eight and in none of the samples was rPL present, at least at levels above 25 ng/ml.

A final attempt to detect rPL in the circulation following injection of solutions containing rPL was done by administering the solution via the carotid cannula. In this case either incubation media containing rPL (3070 ng) from Day 11 placentas or serum from Day 11 pregnant rats containing 1900 ng rPL were used in long-term hysterectomized rats. As can be seen in Fig. 3, both injections resulted in easily measurable amounts of rPL within 10 min after injection. Plasma levels of rPL declined gradually, were still present at 2 hr, but absent at 24 hr. Assuming a plasma volume of 12 ml (300 g body wt $\times$ 4%), it is reasonable that an injection of 3070 ng would result in levels slightly above 100 ng/ml. When the amount of rPL injected

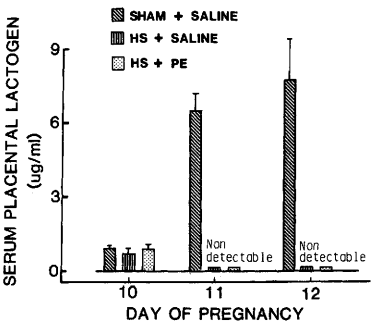

FIG. 2. Serum rPL levels in hysterectomized (HS) rats injected with placental extracts (PE). See Fig. 1 for details of experiment. Serum rPL levels were undetectable following HS and injection of PE.

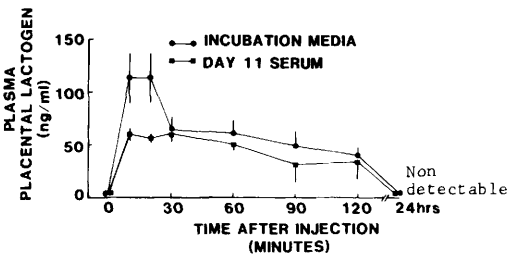

FIG. 3. Plasma rPL levels in long-term hysterectomized rats following injection of incubation media (3070 ng) or serum from Day 11 rats (1900 ng). n = 6 for media and 4 for serum-injected rats.

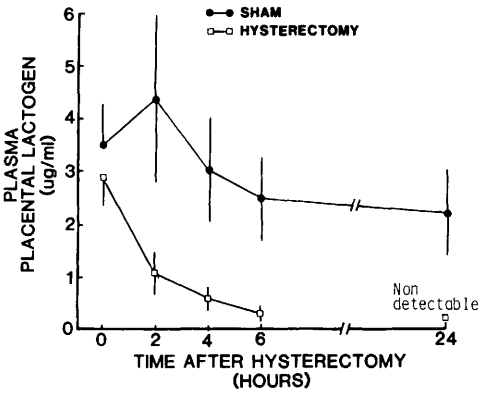

FIG. 4. Plasma rPL levels following hysterectomy in Day 11 pregnant rats. n = 7 for Sham group and 6 for HS group.

was less (1900 ng), the plasma level of rPL at 10 min was less.

From these experiments it is clear why attempts to demonstrate an inhibitory feedback effect of rPL on prolactin *in vivo* by injecting solutions containing rPL have failed. The amount of rPL that reaches the hypothalamic pituitary axis is very little or none when injections are given ip. Even when given via the carotid cannula, the circulating levels did not approach that seen in a normal intact pregnancy (2000–3000 ng/ml). In order for one to conclude that rPL does or does not inhibit prolactin *in vivo,* one must devise a method to maintain high circulatory rPL levels for a period of several hours. Still unanswered is why injection of solutions containing rPL intraperitoneally did not result in the presence of rPL in the blood. The amount of rPL injected was able to maintain the corpora lutea in hypophysectomized pseudopregnant rats (5). Perhaps some rPL reaches the ovary via the peritoneal cavity, but very little is taken up by the capillaries.

Another aspect of this problem is how rapidly rPL is cleared from the circulation following hysterectomy of pregnant rats. Since the peak levels of rPL I occur on Day 11 or 12 and rPL II around Day 17 of pregnancy, rats were hysterectomized at those two times following placement of a cannula in the carotid for blood sampling. Fig. 4 shows the data for Day 11 rats bled at relatively long intervals. The sham-operated rats showed some random variability in rPL levels during the first 6 hr and at no time were the mean values different from each other. Although the rPL value at 24 hr (Day 12) appeared lower than on Day 11, this was not significant. Hysterectomy resulted in a continual decline in rPL such that at 24 hr none was detectable (Fig. 4). When this experiment was repeated on Day 17 of pregnancy, no rPL was detected 2 hr after hysterectomy, even though the mean value at zero time in 11 rats was 2618 ng/ml. However, plasma prolactin levels were significantly higher 24 hr after hysterectomy compared to sham controls.

In order to get a more accurate description of the disappearance curves for rPL, these experiments were repeated but blood sampling was done at more frequent intervals. Fig. 5 shows these data for rats hysterectomized on Day 11. There is a rapid decline in rPL levels during the first 10 min, followed by a plateau for 90 min. By 2 hr there is a further decline, similar to what is shown in Fig. 4. Thus it appears that rPL I has 2 components in its disappearance curve. There

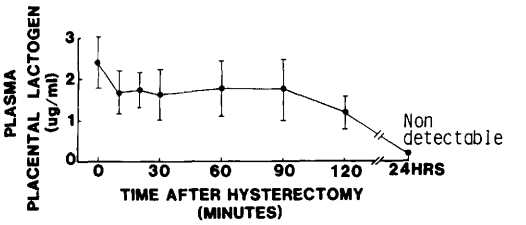

FIG. 5. Plasma rPL levels following hysterectomy in Day 11 pregnant rats bled at frequent intervals (n = 7).

is an initial rapid fall the first 10 min, followed by a gradual decline the next 4–6 hr. The same experiment was repeated on Day 17 of pregnancy. Plasma rPL levels at 10 hr were 22% and at 20 hr were 7% of time zero values. By 30 min posthysterectomy almost all rPL had been cleared from the circulation.

Another report of the disappearance of rPL is that of Kelly *et al.* (11) who measured rPL using a rabbit mammary gland receptor assay. They found a plasma $t_{1/2}$ of 19.5 min for Day 12 and 1.2 min for Days 17–21 pregnant rats. What they missed because they only carried out their measurements for 30 min is that rPL I (Day 11) remains present for several hours after hysterectomy (Fig. 4). A recent abstract reported that the $t_{1/2}$ for rPL I has two components; 30 and 732 min (12). Although our findings do not agree that the second component is that long, they do indicate it is much more than 19 min.

In summary, this study suggests that an intraperitoneal injection of solutions containing rPL does not result in inhibition of prolactin *in vivo* because very little of the injected rPL is later found in the circulation. This may be due to poor absorption of rPL by capillaries in the peritoneal cavity or rapid destruction by the liver. It is not likely that rPL reaches the blood and is rapidly destroyed, since hysterectomy on Day 11 of pregnancy does not result in rapid disappearance of rPL from the circulation. Even though a direct inhibitory effect of rPL on prolactin secretion from the pituitary *in vitro* has been reported (13), any demonstration of such an effect *in vivo* will first require a method to continuously infuse large amounts of rPL intravenously in order to sustain the high levels of rPL seen during pregnancy. Thus the hypothesis that the midgestational rise in rPL shuts off prolactin secretion is supported only by the correlative data showing an inverse relationship between rPL and prolactin levels. Direct demonstration of an inhibitory effect of rPL *in vivo* requires further experimentation.

1. Yogev L, Terkel J. Timing of termination of nocturnal prolactin surges in pregnant rats as determined by the number of fetuses. J Endocrinol **84**:421–424, 1980.
2. Voogt J, Robertson M, Friesen H. Inverse relationship of prolactin and rat placental lactogen during pregnancy. Biol Reprod **26**:800–805, 1982.
3. Yogev L, Terkel J. Endogenous inhibition of prolactin secretion in pregnant lactating rats. Endocrinology **110**:158–162, 1982.
4. Tonkowicz PA, Voogt JL. Termination of prolactin surges with development of placental lactogen secretion in the pregnant rat. Endocrinology **113**:1314–1318, 1983.
5. Smith MS, Neill JD. Termination at mid-pregnancy of the two daily surges of plasma prolactin initiated by mating in the rat. Endocrinology **98**:696–701, 1976.
6. Voogt JL. Regulation of nocturnal prolactin surges during pregnancy in the rat. Endocrinology **106**: 1670–1676, 1980.
7. Yogev L, Gibber JR, Terkel J. Failure of rat placenta to inhibit prolactin secretion by ectopic pituitary gland. J Endocrinol **97**:91–95, 1983.
8. Matthies DL. Studies of the luteotropic and mammotropic factor found in trophoblast and maternal peripheral blood of the rat at mid-pregnancy. Anat Rec **159**:55–68, 1967.
9. Tanaka T, Shiu RPC, Gout PW, Beer CT, Noble RT, Friesen HG. A new sensitive and specific bioassay for lactogenic hormones: Measurement of prolactin and growth hormones in human serum. J Clin Endocrinol Metab **51**:1058–1063, 1980.
10. Tonkowicz PA, Voogt JL. Examination of rat placental lactogen and prolactin at 6 hr intervals during midpregnancy. Proc Soc Exp Biol Med **173**:583–587, 1983.
11. Kelly PA, Shiu RPC, Robertson MC, Friesen HG. Characterization of rat chorionic mammotropin. Endocrinology **96**:1187–1195, 1975.
12. Glaser LA, Khan MI, Pepe GJ, Kelly PA, Gibori G. Differential action and clearance of rat placental lactogens. IV International Congress on Prolactin, Abstract No 100, 1984.
13. Voogt JL. Evidence for an inhibitory influence of rat placental lactogen on prolactin release in vitro. Biol Reprod **31**:141–147, 1984.

Received October 16, 1984. P.S.E.B.M. 1985, Vol. 178.
Accepted December 4, 1984.