PROLACTIN AND LUTEINIZING HORMONE LEVELS BEFORE AND AFTER PARTURITION IN RATS BEARING EITHER SINGLE OR MULTIPLE EMBRYOS

I. NIR*, G. GOLDBHABER, N. HIRSCHMANN AND J. SHANI

Department of Applied Pharmacology, School of Pharmacy, The Hebrew University of Jerusalem, P.O.B. 12065, Jerusalem, Israel

(Received 12 June 1976)

SUMMARY

Levels of prolactin and LH were determined in serum and pituitary during the last days of pregnancy and post partum in rats bearing single and multiple embryos. In rats with a single embryo serum prolactin and LH levels were significantly lower during the last 2 days of pregnancy and post partum than in rats bearing multiple embryos. While large increases were recorded in serum prolactin and LH levels in the rats with multiple embryos between days 21 and 22 of gestation, in the group with single embryos changes occurred in LH level only. Throughout the experiment pituitary prolactin was lower in rats with a single embryo than in those with multiple embryos in spite of the sharp drop in prolactin level in the group with multiple embryos from day 21 to 22. No differences were observed in the pituitary LH levels of either group during the days preceding parturition, but in the rats with multiple embryos there was a sharp drop in LH level post partum. It seems that the reduced serum prolactin level in the rats with a single embryo was associated with inhibition of pituitary prolactin synthesis and release, whereas the decreased serum LH level resulted from impaired release but not synthesis. These results support the hypothesis of a regulatory role for the placenta in pituitary prolactin and LH synthesis and release, either by hypothalamus–pituitary stimulation, or perhaps by way of the ovaries, through regulation of ovarian steroid production.

INTRODUCTION

During the last days of pregnancy a marked increase in serum prolactin and a corresponding decrease in pituitary prolactin has been demonstrated in the rat (Amenomori, Chen & Meites, 1970; Bast & Melampy, 1972; Morishige, Pepe & Rothschild, 1973). This follows high pituitary and low serum prolactin levels during days 4–20 of pregnancy and is succeeded by a marked decrease in serum prolactin after parturition (Bast & Melampy, 1972).

Similarly, serum luteinizing hormone (LH) levels increase from day 21 of gestation until term accompanied by a decrease in progesterone in the blood, presumably due to regression of corpora lutea, while follicle-stimulating hormone levels in serum decrease after day 18. In the pituitary there is a dramatic increase in LH content up to day 10 of pregnancy, but thereafter it remains relatively constant until parturition (Bast & Melampy, 1972; Linkie & Niswender, 1972; Morishige et al. 1973).

The factors regulating pituitary synthesis and release of LH and prolactin during the final phase of pregnancy have not yet been defined. Bridges & Goldman (1975) ascribe the

* Requests for reprints should be addressed to Professor I. Nir.
increased serum prolactin to ovarian stimulation; Vermouth & Deis (1974) contend that pituitary release is under an inhibitory influence of sex hormones, while others (Simpson, Simpson & Kulkarni, 1973) suggest that the increase in serum prolactin during late pregnancy is independent of ovarian factors.

Pituitary prolactin loses its importance in maintaining pregnancy about 10 days after mating and it was considered possible that in rodents this function may be taken over by the placenta through the secretion of lactogen (Ray, Averill, Lyons & Johnston, 1955; Kohimoto & Bern, 1970). Moreover, it is believed that after day 11 of gestation, the placenta may play a role in the regulation of ovarian steroid production (Astwood & Greep, 1938; Averill, Ray & Lyons, 1950; Matthis, 1967).

The possible influence of the placenta on regulation of prolactin and LH during the last days of pregnancy and immediately after parturition has been studied by comparing pituitary contents and serum levels of prolactin and LH in rats bearing single or multiple embryos.

**MATERIALS AND METHODS**

Two hundred and ten female albino rats of the Hebrew University ‘Sabra’ strain, weighing 180–220 g each, were maintained on a lighting schedule of 12 h light:12 h darkness and supplied with food and water *ad libitum*. Vaginal smears were taken daily and the animals were mated on the day of pro-oestrus. Day 1 of gestation was designated as the day on which a vaginal plug or sperm was found in the oestrous smear.

One hundred and fifty-seven pregnant rats were divided into two groups: in the first group, all embryos except one were removed from each rat through a mid-ventral incision under ether anaesthesia on day 12 of pregnancy; the second group was left intact. Starting on day 21 of gestation and continuing until day 1 after parturition, rats were killed each day between 10.00 and 11.00 h (except after delivery when decapitation was carried out between 2 and 4 h post partum) and their blood and pituitary glands were collected.

To separate the pups from their mothers and prevent suckling, 2 × 12 cm gauge netting was inserted 3 cm above the bases of the cages of rats kept until delivery.

Blood samples were stored overnight at 4 °C and then centrifuged at 1200g. Pituitary glands were weighed and homogenised in 0-5 ml 0-01 m-NaOH solution and the homogenates diluted to 10 ml with a phosphate diluent (pH 7.6) containing 0-5 ml bovine serum albumin and centrifuged at 1200g. Sera and pituitary extracts were frozen at –20 °C.

Prolactin and LH contents were expressed in terms of NIAMDD rat prolactin-RP-1 (11-0 i.u./mg) and rat LH-RP-1 (0-03 × NIH-LH-S1) respectively. All sera and pituitary extracts were assayed in two dilutions and in triplicate and the means evaluated according to Student’s *t*-test.

Sham-operation was performed on 50 pregnant rats on day 12 of gestation. On days 21 or 22 after conception, or post partum, at least 14 of the animals were killed and their serum and pituitary LH and prolactin levels were determined. The levels of serum and pituitary prolactin and LH in the sham-operated animals were identical to those obtained in the intact rats with multiple embryos.

**RESULTS**

**Prolactin**

Serum prolactin levels in rats bearing multiple embryos doubled from day 21 to 22 of pregnancy (*P* < 0.005) and dropped abruptly (*P* < 0.01) after parturition. In rats with single embryos no changes in serum prolactin levels were observed during days 21–23 of pregnancy or *post partum*. All values, however, were significantly lower than the levels in rats bearing multiple embryos (*P* < 0.05) (Table 1).
Serum prolactin and LH in rats

Table 1. Serum and pituitary prolactin levels in rats bearing multiple and single embryos on days 21–23 after conception and post partum (means ± S.E.M.)

<table>
<thead>
<tr>
<th>Days after conception</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>2-4 h post partum†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum prolactin (ng RP-1/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple-embryo rats</td>
<td>72.8 ± 5.1 (21)</td>
<td>134.0 ± 14** (17)</td>
<td>—</td>
<td>83.7 ± 9.9* (14)</td>
</tr>
<tr>
<td>Single-embryo rats</td>
<td>45.6 ± 2.9†† (17)</td>
<td>51.0 ± 5.5†† (18)</td>
<td>56.0 ± 6.5 (18)</td>
<td>60.0 ± 3.8† (17)</td>
</tr>
<tr>
<td>Pituitary prolactin (µg RP-1/gland)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple-embryo rats</td>
<td>18.7 ± 0.7 (33)</td>
<td>10.4 ± 0.6*** (36)</td>
<td>—</td>
<td>10.8 ± 0.8 (15)</td>
</tr>
<tr>
<td>Single-embryo rats</td>
<td>6.7 ± 0.4†† (15)</td>
<td>7.7 ± 0.5†† (17)</td>
<td>8.3 ± 0.6 (18)</td>
<td>8.1 ± 0.3† (14)</td>
</tr>
</tbody>
</table>

* P < 0.01;  ** P < 0.005;  *** P < 0.001: significance of differences within each group between consecutive days.
† P < 0.05;  †† P < 0.005: significance of differences between the multiple- and single-embryo groups on the same days.
‡ Parturition occurred on day 23 in rats bearing multiple embryos.
Number of animals in parentheses.

Pituitary prolactin levels in the rats with multiple embryos decreased by about 50% from day 21 to 22, but no change was observed post partum. In the rats with single embryos no changes took place in pituitary prolactin levels, but all levels were significantly lower (P < 0.01) compared with those of rats with multiple embryos (Table 1).

Luteinizing hormone

Serum LH levels in rats bearing multiple embryos increased from day 21 to 22 of pregnancy (P < 0.001) and decreased post partum (P < 0.05), while in the rats with single embryos a steady increase in serum LH concentration occurred from day 21 of gestation, reaching a high significance post partum (P < 0.005) (Table 2). During the days preceding parturition (21 and 22) the serum LH levels in the single embryo group were significantly lower than those of the multiple embryo rats and remained so after parturition.

Table 2. Serum and pituitary LH levels in rats bearing multiple and single embryos on days 21–23 after conception and post partum (means ± S.E.M.)

<table>
<thead>
<tr>
<th>Days after conception</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>2-4 h post partum†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum LH (ng RP-1/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple-embryo rats</td>
<td>104.0 ± 6.1 (21)</td>
<td>130.0 ± 3.5*** (21)</td>
<td>—</td>
<td>117.0 ± 4.6* (14)</td>
</tr>
<tr>
<td>Single-embryo rats</td>
<td>60.0 ± 2.9†† (16)</td>
<td>77.1 ± 7.2††* (15)</td>
<td>87.4 ± 3.6 (14)</td>
<td>102.0 ± 3.3†** (13)</td>
</tr>
<tr>
<td>Pituitary LH (µg RP-1/gland)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple-embryo rats</td>
<td>892 ± 43 (19)</td>
<td>904 ± 27 (20)</td>
<td>—</td>
<td>608 ± 27*** (14)</td>
</tr>
<tr>
<td>Single-embryo rats</td>
<td>839 ± 42 (17)</td>
<td>847 ± 41 (15)</td>
<td>867 ± 46 (15)</td>
<td>938 ± 52†† (12)</td>
</tr>
</tbody>
</table>

* P < 0.05;  ** P < 0.005;  *** P < 0.001: significance of differences within each group between consecutive days.
† P < 0.05;  †† P < 0.005: significance of differences between multiple- and single-embryo groups on the same days.
‡ Parturition occurred on day 23 in rats bearing multiple embryos.
Number of animals in parentheses.

The expected decrease in pituitary levels in rats bearing multiple embryos between days 21 and 22 did not occur, but a marked decrease occurred from day 22 until after parturition. There were no changes in pituitary LH levels in rats bearing single embryos during either
the days before or after parturition. Moreover, there were no detectable differences between the pituitary LH levels of the two groups until a sharp drop took place in pituitary LH content of the rats bearing multiple embryos post partum (Table 2).

Pregnancy was prolonged in those rats bearing a single embryo, being 24±14±0.19 (s.e.m.) days as compared with 22.69±0.18 days in the rats with multiple embryos (P < 0.005).

**DISCUSSION**

We were prompted into our search for alternative regulatory factors for prolactin and LH production and release during the final phase of pregnancy by the contradictory interpretations of results on the role of the ovaries presented by various workers (Simpson et al. 1973; Morishige et al. 1973; Vermouth & Deis, 1974; Bridges & Goldman, 1975). Although Bridges & Goldman (1975) found clear indications that the rise in serum prolactin occurring during late pregnancy in the rat is under ovarian influence, as they themselves pointed out, their study did not exclude the possibility that extra-ovarian sources may be affecting the prolactin release. The fact that even after removal of the pituitary on day 11 of gestation pregnancy continued until term (Pencharz & Long, 1933), suggested that extra-pituitary factors could be responsible for the luteal function of late pregnancy (Astwood & Greep, 1938; Linkie & Niswender, 1971). In the present study definite differences in prolactin and LH concentrations of rats bearing single and multiple embryos were recorded during late pregnancy. The main differences observed were in the serum levels of prolactin and LH which were much lower during days 21 and 22 of gestation in rats with a single embryo than in those with several. Since animals with a single embryo and placenta were found to produce significantly lower serum levels of prolactin and LH than animals bearing multiple embryos and placentae, the conceptus (placenta plus embryo) may be involved in controlling prolactin and LH in late pregnancy.

The lower serum and pituitary prolactin levels seen in rats with a single embryo as compared with those bearing multiple embryos, indicate decreased synthesis and release of pituitary prolactin. In the case of LH the lower levels observed in rats with a single embryo were found only in serum, the pituitary concentration remained steady and equal during the entire pre-parturition period compared with that of rats carrying multiple embryos. This finding, plus the fact that after parturition the concentration of LH in the pituitary was found to be decreased only in the group carrying multiple embryos but tended to be increased in the rats bearing a single embryo led us to conclude that the changes in LH of the latter are associated with decreased release, but not synthesis, of the gonadotrophin. Attempts in the past to demonstrate a gonadotrophic substance in the placentae of rats were unsuccessful. Placental extracts, however, when injected into normal adult females beginning on the day of oestrus, inhibit cycles and it was concluded that the active material in rat placenta is unlike any known gonadotrophic substance (Astwood & Greep, 1938). The ability of the placenta to support deciduomata in hypophysectomized mature pseudopregnant rats led Linkie & Niswender (1971, 1972, 1973) to accept this as an indication of a luteotrophic factor present in the placenta during pregnancy.

The possibility that the effect of the conceptus may be exerted through the ovaries, by control of either oestrogen or progesterone production is not excluded. Oestrogen levels tend to be higher in rats bearing single embryos (preliminary findings in our laboratory) and this would indicate that prolactin synthesis and release may be controlled by oestrogen, which increases markedly during the last days of pregnancy (Shaikh, 1971; McCormack & Greenwald, 1974). This would be in accord with the results of Vermouth & Deis (1974), who found decreased serum prolactin levels in ovariectomized pregnant rats treated with oestradiol. In addition, the possibility exists that the prolactin and LH increase is triggered
by a decrease in serum progesterone. A dramatic decrease in ovarian venous progesterone levels, beginning on day 18 of gestation, has been reported by Fajer & Barraclough (1967). Morishige et al. (1973) showed a correlation between serum progesterone decrease and serum prolactin increase during the last days of pregnancy, and progesterone treatment prevented the rise in prolactin levels observed 4 and 8 h after ovariectomy and also inhibited oestrogen-stimulated prolactin release (Vermouth & Deis, 1974).

In our studies, when pups were prevented from sucking, post-partum levels of serum prolactin were markedly decreased. This supports Bast & Melampy's (1972) finding of decreased serum prolactin shortly after delivery.

As to the delayed parturition which usually took place in rats bearing single embryos, it is not very likely that the number of foetuses plays a role in the control of parturition in the rat, since when all foetuses were removed and the placentae left in situ, delivery of the placentae tended to occur at term (Newton, 1935). In fact an inverse relation between duration of gestation and litter size in mice was noted by Biggers, Curnow, Finn & McLaren (1963). In addition, treatment of pregnant rats with LH did not advance parturition and treatment with anti-LH did not delay it (Madhwa Raj & Moudgal, 1970). No reports of investigations on the effect of prolactin on timing of parturition are to be found in the literature.

These results support the hypothesis that the placenta plays a regulatory role in pituitary prolactin and LH synthesis and release, either in addition to ovarian stimulation, or indirectly, through regulation of ovarian steroid production.

We wish to thank Miss Ute Schmidt for her invaluable technical assistance, the Joint Research Fund of the Hebrew University and Hadassah Medical Organization for financial support, and NIAMDD for the generous gift of rat prolactin and LH radioimmunoassay kits.

REFERENCES


