MAMMOTROPHIC ACTIVITY IN RAT AMNIOTIC FLUID

By

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ABSTRACT

Mammotrophic activity was present in rat amniotic fluid collected on days 13, 15 and 19 of pregnancy. The mammotrophic activity in the fluid was equivalent to that in the maternal serum on day 15, but it was lower on day 19. The effect of the amniotic fluid was similar to that of human placental lactogen on the rat mammary gland in vitro. The prolactin concentration in the amniotic fluid was low (approximately 1 ng/ml).

The possibility of mammotrophic activity being present in rat amniotic fluid, has previously been explored by Shani et al. (1970). They collected amniotic fluid from 18–20-day pregnant rats and determined its pigeon crop stimulating effect and the degree of mammary development that could be produced in rats primed with oestradiol. No activity could be demonstrated. However, a preliminary experiment in this laboratory, using an organ culture technique, demonstrated mammotrophic activity in rat amniotic fluid, collected on day 15 of pregnancy.

The present study confirms this observation. Furthermore, mammotrophic activity of rat amniotic fluid was compared with the activity present in the corresponding maternal serum and with the effect of human placental lactogen (hPL) on rat mammary gland in vitro. In addition the concentration of prolactin in the amniotic fluid was determined.
MATERIALS AND METHODS

Amniotic fluid, serum and medium. – Nulliparous rats of the random bred Cpb: WU (WI) strain (derived from the Wistar strain) were mated. The day on which the vaginal smear showed the presence of spermatozoa, was considered as day 0 of pregnancy. Amniotic fluid was obtained by opening the amniotic cavity under sterile conditions and collecting the escaping fluid in a sterile tube. The fluid was centrifuged for 20 min (1400 g), the clear supernatant collected and centrifuged for another 5 min (1400 g). The final samples were stored at -20°C. Serum samples were collected from the same rats. Details of collection and preparation of the serum samples are described elsewhere (Peters et al. 1976).

The medium consisted partly of a chemically defined medium (t8), a simplified formula adapted from Trowell’s T8 (Paul 1970) i.e. without amino acids, vitamins or phenol red. The final glucose concentration in the medium was approximately 2 mg/ml. The medium was supplemented with insulin (50 μg/ml; 25.7 IU/mg; Organon, Oss, The Netherlands).

Culture. – The mammotrophic activity was measured in mammary gland explants of 13 days pregnant rats. The technique has been described previously (Peters et al. 1976). The glands of 4 rats were used in each of the 3 days cultures, except in exp. 3 (1 rat).

Histology and grading of the results are described elsewhere (Peters et al. 1976). The histological end-points which were important in these experiments, were mitotic activity, cytoplasmic opalescence and degree of alveolar development. Differences between groups were tested for statistical significance by the distribution-free test of Wilcoxon (Wabeke & van Eeden 1970) or the chi-square test (Croxton 1959). A level of significance of 5% or 1% was chosen.

Radioimmunoassay. – The prolactin levels of the amniotic fluid of three 15 days and four 19 days pregnant rats were determined by the method described by Kwa et al. (1972).

Experiments

The following samples were assayed:

Exp. 1: Serum and amniotic fluid collected on days 15 and 19 of pregnancy. – In the first set of experiments, amniotic fluid was added to the medium in the range of 0.4 to 6.3%, in a second set of experiments 1.6 to 25%. The culture medium had the following composition: 50% t8 +25% pooled virgin rat serum +25% consisting of various concentrations of amniotic fluid complemented by appropriate quantities of t8, from which, however, the glucose was omitted.

The mammotrophic activity of the sera, tested in the same range of concentrations as the samples of amniotic fluid, was assayed in a medium containing 50% t8 +25% t8 without glucose + pregnant rat serum in various concentrations to which was added pooled virgin rat serum to make a final concentration of 25% serum.

Exp. 2: Comparison between amniotic fluid collected on day 15 of pregnancy and hPL at various concentrations. – Amniotic fluid collected from a 15 days pregnant rat was added to the medium in concentrations of 0.8 to 25%. The composition of the medium was as used in exp. 1.

Human placental lactogen (1 mg hPL/ml distilled water, preparation of Dr. H. G.
Friesen, Montréal, Canada) was added after millipore sterilization to the medium at 0.08, 0.31, 1.25 and 5 µg/ml medium. The medium consisted of 50% t8 + 25% t8 without glucose + 25% virgin rat serum.

Exp. 3: Amniotic fluid collected on day 13 of pregnancy. — Mammotrophic activity of amniotic fluid collected from a 13 days pregnant rat was measured in a medium consisting of 25% amniotic fluid + 50% t8 + 25% virgin rat serum. It was compared with a medium in which the amniotic fluid was replaced by 25% t8 without glucose.

RESULTS

1. The mammary gland of 13 days pregnant rats

The morphology of the material collected at the onset of the experiment and subsequently used for the organ culture, has been described elsewhere (Peters et al. 1976).

Effect of amniotic fluid (AF) and serum (S) collected from a 15 days and from a 19 days pregnant rat, on rat mammary gland in vitro (exp. 1). Mitotic activity and cytoplasmic opalescence in the acini are expressed as percentage of fragments reaching grade 2 or more by semiquantitative grading. Three days culture with insulin supplementation. Results obtained with virgin rat serum without AF are indicated by horizontal arrows. The concentrations at which the results with AF or S became significantly different from control 0% are indicated for mitotic activity or cytoplasmic opalescence by pointers. Significant differences between the result obtained with AF (---) or S (-----) at various concentrations are indicated by one (at the 5% level) or two asterisks (at 1% level). Number of explants per group: 12.

376
Mitotic activity
day 15  day 19
cytoplasmic opalescence
day 15  day 19

% serum or amniotic fluid

Fig. 2.
Effect of amniotic fluid (AF) and serum (S) collected from a 15 days and a 19 days pregnant rat, on rat mammary gland *in vitro* (exp. 1). For explanation: see Fig. 1.

Table 1.
Effect of amniotic fluid (AF) and serum collected from 15 days and from 19 days pregnant rats, on the alveolar development of rat mammary gland *in vitro* (exp. 1). The degree of alveolar development is expressed as median grade. Significant differences between the results obtained with AF or serum at various concentrations are indicated by one (at the 5% level) or two asterisks (at 1% level). Number of explants per group: 12.

<table>
<thead>
<tr>
<th>% in medium</th>
<th>Rat 1</th>
<th></th>
<th>Rat 2</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>AF</td>
<td>Serum</td>
<td>AF</td>
<td>Serum</td>
</tr>
<tr>
<td></td>
<td>Day 15</td>
<td>Day 19</td>
<td>Day 15</td>
<td>Day 19</td>
</tr>
<tr>
<td>0</td>
<td>3-4</td>
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<td>4</td>
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<td>0.4</td>
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<td>3</td>
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<td>4</td>
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<td>4</td>
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<tr>
<td>25</td>
<td>5**</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>
2. Mammatrophic activity in amniotic fluid and serum (exp. 1)

Figs. 1 and 2 show the results obtained with amniotic fluid and serum added to the medium in the ranges of 0.4 to 6.3% and of 1.6 to 25%. Both the amniotic fluid and the serum were collected from rats at day 15 and day 19 of pregnancy, respectively. Compared with the medium without any pregnant rat serum or amniotic fluid, the mitotic activity and the cytoplasmic opalescence increased with increasing concentrations of either pregnant rat serum or amniotic fluid. This tendency was less apparent for the alveolar development (Table 1). The concentrations at which the difference with the medium without pregnant rat serum or amniotic fluid became statistically significant are indicated in Figs. 1 and 2.

At day 15 of pregnancy the mammotrophic activity of the amniotic fluid was equivalent to the activity in the serum. At day 19, however, the mammotrophic activity in the amniotic fluid was consistently lower than the activity in the serum. The activity of the serum was approximately four times that of the amniotic fluid. The sera of the two 19 days pregnant rats were more active than the sera of the two 15 days pregnant rats; the reverse was the case with the amniotic fluids.

![Graph showing effect of amniotic fluid and hPL](https://via.placeholder.com/150)

**Fig. 3.** Effect of the amniotic fluid of a 15 days pregnant rat and hPL at various concentrations on rat mammary gland in vitro (exp. 2). Numbers (top) indicate the median grade for alveolar development. Mitotic activity (---) and cytoplasmic opalescence (----) are expressed as % of fragments reaching grade 2 or more by semiquantitative grading. Three days culture with insulin supplementation. Results obtained with virgin rat serum without amniotic fluid or hPL are indicated by horizontal arrows and the corresponding median grade for development. Number of explants per group: 12.
Table 2.
Effect of amniotic fluid (25% of the medium) collected from a 13 days pregnant rat, on rat mammary gland in vitro. Three days culture with insulin supplementation.

<table>
<thead>
<tr>
<th>Amniotic fluid in the medium</th>
<th>Alveolar development grade*</th>
<th>Mitotic activity %**</th>
<th>Cytoplasmic opalescence %***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>5</td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>Absent</td>
<td>3</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

* Medium value of semiquantitative grading of response indicated.
** % of explants showing response indicated.

No. of explants: 12 per group. Differences between the two groups significant at 1 % level for all three parameters.

3. Mammotrophic activity of amniotic fluid compared with the effect of hPL (exp. 2)

Fig. 3 shows the results obtained with various concentrations of hPL and amniotic fluid collected on day 15 of pregnancy. In both series an increase of alveolar development, mitotic activity and cytoplasmic opalescence with increasing concentrations could be observed. A rough estimate indicates that the activity present in 1 ml amniotic fluid on day 15 of pregnancy is equivalent to approximately 50 to 100 μg of the hPL preparation.

4. Mammatrophic activity of amniotic fluid on day 13 of pregnancy (exp. 3)

Table 2 shows that mammatrophic activity was also present in the amniotic fluid collected from a 13 days pregnant rat.

5. Prolactin level in the amniotic fluid on days 15 and 19 of pregnancy

The prolactin concentration in the amniotic fluid of 15 days pregnant rats was 1.0 ± 0.06 ng/ml (mean ± sd, n = 3) and of 19 days pregnant rats 1.1 ± 0.05 ng/ml (mean ± sd, n = 4).

DISCUSSION

Rat amniotic fluid exerts mammatrophic activity on the mammary gland in vitro. This activity is demonstrated to be present in samples collected on days 13, 15 and 19 of pregnancy. This cannot be explained by the presence of prolactin in the amniotic fluid, since the levels of this hormone are very
low. The possibility of high levels of growth hormone has not been excluded, but the explanation most likely for the mammotrophic activity seems to be the presence of rat chorionic mammotrophin (rCM) in the fluid. This hormone is present in rat serum from day 8 to 9 of pregnancy till parturition (Kelly et al. 1975).

The varying degrees of mammotrophic activity demonstrated both in serum and amniotic fluid by organ culture are not strictly comparable because of a difference in the composition of the medium in which serum and amniotic fluid were tested, but otherwise the mammotrophic activity in the amniotic fluid appeared to be equivalent to the activity found in the maternal serum on day 15 of pregnancy. On day 19 the activity in the amniotic fluid was less than in the maternal serum. The possibility exists that rCM is secreted directly by certain placental elements into the amniotic fluid and does not originate from the maternal circulation. The lower levels of activity found in the amniotic fluid in late pregnancy can be the result of a decrease in the rate of secretion or a decrease of secreting cells relative to the amount of amniotic fluid.

The effect of the amniotic fluid on the mammary gland in vitro is similar to the effect which can be obtained with hPL. However, the concentration of the hPL preparation necessary to obtain a comparable degree of effect is too high to assume a similar concentration of rCM to be present in amniotic fluid. It is possible that the potency of the hPL preparation is low in the system used or that the assay method is not sufficiently precise (Forsyth & Parke 1973).

Shani et al. (1970) were unable to demonstrate mammotrophic activity in rat amniotic fluid. The discrepancy between the results of those investigators and the results reported in the present paper may be due to differences in the assay-technique. The mammotrophic activity in rat serum during the last week of pregnancy was not detected by an in vivo technique (Matthies 1968; Cohen & Gala 1969), but could be demonstrated by radioreceptor assay (Kelly et al. 1975) and by organ culture (Peters et al. 1976).

rCM may possess some prolactin- and growth hormone-like properties. The significance for the foetus of high levels of this hormone in the amniotic fluid remains a matter of speculation.

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REFERENCES


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