Quantitative Participation of Placental Mammotropic Hormones in Mammary Development During Pregnancy of Mice

HIROSHI NAGASAWA AND REIKO YANAI
Pharmacology Division, National Cancer Center Research Institute, Tsukiji 3-1-1, Chuo-ku, Tokyo

Synopsis

In order to elucidate the importance of the biological role of placental mammotropic hormones in the mammary development during pregnancy, the correlations between the number and weight of placentae and some indices of mammary development were examined using nulliparous C3H/He pregnant mice. On day 8 of pregnancy, the number of placentae was adjusted to 1–12 each by removing surgically the placentae with fetuses and all the mice were killed on day 19 of pregnancy. DNA and RNA contents and RNA/DNA ratio in bilateral inguinal mammary glands and lobuloalveolar rating of the right third thoracic gland were employed as the indices of mammary development. Positive and statistically highly significant correlations were obtained between both the number and weight of placentae and each index of mammary development, and the number and weight of placentae had higher correlations with RNA and RNA/DNA than with DNA. On the other hand, pituitary levels of prolactin and growth hormone had no correlations with any index of mammary development. These results provide evidence that placental mammotropic hormones contribute quantitatively to the mammary development during pregnancy in mice and suggest that the lactogenic effect of placental mammotropic hormones would be more prominent than their mammogenic effect at late pregnancy.

The existence of mammotropin (prolactin-like substance) in the placenta has been substantiated in mice (Newton and Beck, 1939; Cerruti and Lyons, 1960; Kohmoto and Bern, 1970; Yanai and Nagasawa, 1971a) as well as in rats (Pencharz and Long, 1933; Ray et al., 1955; Matthies, 1967). Pregnancy can be maintained and mammary development can proceed in these species after hypophysectomy provided that placental mammotropin is present together with steroid hormones (Pencharz and Long, 1931; Newton and Beck, 1939; Averill et al., 1950). Recently, Yanai and Nagasawa (1971b) found in C3H/He and C57BL/6 strains of pregnant mice that pituitary prolactin levels changed little or decreased gradually with the advance of pregnancy, whereas mammary glands continued to grow throughout this period. An interest in these investigations prompted testing the quantitative relationships between the number and weight of placentae and mammary development as one step to ensure a more precise definition of biological property of placental mammotropic hormones in the mammary development during pregnancy.

Materials and Methods

C3H/He female mice bred in our laboratory were used. They were maintained in an air-conditioned (24 ± 0.5°C in temperature and 65–68% in relative humidity) and artificially illuminated (14 hours’ light from 7:00 AM to 9:00 PM) animal room and given

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commercial diet (CA-1; Japan CLEA Ltd., Tokyo) and tap water ad libitum.

At the age of 80–100 days, they were mated with males and vaginal smears were made every morning (8:00 AM) beginning 7 days before mating. The day when copulation plug or sperm was found or the last estrous day was designated as day 1 of pregnancy. According to this criterion, almost all mice not killed have been found to deliver young on the early morning of day 20 of pregnancy. On day 8 of pregnancy, the number of placentae was adjusted to 1–12 per mouse by removing placentae with fetuses surgically under

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**Fig. 1.** Correlations between number and weight of placentae and indices of mammary development in mice on day 19 of pregnancy.

Number of placentae was adjusted surgically on day 8 of pregnancy. All the correlation coefficients were statistically significant at P < 0.001 (*P < 0.05).

$r'$: Correlation coefficients in the case that the data of mice with more than 9 placentae (○) were excluded.

( ) : Number of samples examined.
light ether anesthesia. All the mice were killed by
decapitation on day 19 of pregnancy and the anterior
pituitary, ovaries, fetuses and placentae were removed
and weighed. The pituitary was immediately frozen
and stored at $-20^\circ$C for assay of prolactin and growth
hormone (GH). The pituitary hormone levels were
determined by disc electrophoresis on polyacrylamide
gel (Yanai and Nagasawa, 1971b). The concentration
of polyacrylamide was 10% and current of 4 mA/
column was supplied at pH 9.5. One pituitary was
employed per column. The contents and concentra-
tions of the hormones were expressed in terms of $\mu g$
NIH-P-B$_2$ (prolactin) or $\mu g$ NIH-GH-B$_{12}$ (GH) per
whole pituitary and per mg pituitary, respectively.
Ovaries were fixed in formalin, embedded in paraffin,
cut at 8µ and stained with hematoxylin and eosin.
The bilateral inguinal mammary glands were defatted
and dried with hot alcohol and ether. Nucleic acids
(DNA and RNA) contents in these tissues were deter-
mined by the same procedure as described previously
(Nagasawa et al., 1964). The right third thoracic
mammary gland was used for the whole mount evalua-
tion. The degree of lobuloalveolar formation was rated
from 1 to 7 in increments of 1 by the standard of
Wrenn et al. (1966) with a simplification.

Results

Correlations between number and weight of
placentae and indices of mammary develop-
ment

The results are presented in Figure 1. All
the correlation coefficients between the num-
ber and weight of placentae and indices of
mammary development examined were pos-
itive and statistically highly significant ($P <
0.05$ or $0.001$). Each regression equation con-
tained no statistically significant difference
from linearity and the slope was significantly
different from zero ($P < 0.05$ or $0.01$). These
results clearly indicate that the degree of the
mammary development during pregnancy in
mice was parallel with both the number and
weight of placentae.

Any value as the index of mammary develop-
ment except DNA did not increase with
the increase of the number of placentae in
mice bearing more than 9 placentae each, and
the correlation coefficients between the num-
ber or weight of placentae and RNA or RNA/
DNA became higher in the case that the data
of these mice were excluded.

No significant correlations existed between
the number of placentae and the average
weights of both placentae and fetuses ($r =
-0.12$ and $-0.29$, respectively).

Correlations between prolactin and GH
levels in the anterior pituitary and indices of
mammary development

As shown in Table 1, there were no cor-
relations between any pituitary hormone
level and mammary nucleic acids contents
and mammary lobuloalveolar rating.

The ovaries were found to be prominently
occupied by functional corpora lutea histol-
ogically irrespective of number of placentae
per uterine body.

Discussion

The quantitative contribution of placental
mammatropic hormones to the mammary
development during pregnancy in mice was

Table 1. Correlations between prolactin (PL) and growth hormone (GH) levels in the anterior
pituitary (AP) and indices of mammary development in mice on day 19 of pregnancy

<table>
<thead>
<tr>
<th></th>
<th>DNA (mg)</th>
<th>RNA (mg)</th>
<th>RNA/DNA</th>
<th>Mammary rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP−PL $\mu g$ NIH-P-B$_2$</td>
<td>$+0.04$ (26)</td>
<td>$-0.01$ (26)</td>
<td>$+0.09$ (26)</td>
<td>$-0.11$ (29)</td>
</tr>
<tr>
<td>PL/PL</td>
<td>$-0.04$ (27)</td>
<td>$+0.04$ (27)</td>
<td>$-0.17$ (27)</td>
<td>$-0.28$ (30)</td>
</tr>
<tr>
<td>AP−GH $\mu g$ NIH-GH-B$_{12}$</td>
<td>$+0.04$ (23)</td>
<td>$+0.03$ (24)</td>
<td>$-0.15$ (24)</td>
<td>$-0.30$ (27)</td>
</tr>
<tr>
<td>GH/GH</td>
<td>$-0.05$ (26)</td>
<td>$-0.23$ (25)</td>
<td>$-0.39$ (25)</td>
<td>$-0.24$ (28)</td>
</tr>
</tbody>
</table>

Number of placentae was adjusted surgically to 1−12 each on day 8 of pregnancy.
Number of samples examined was indicated in parentheses.
interpreted from these results that there existed positive and highly significant correlations between the number and weight of placentae and any index of mammary development (DNA, RNA, RNA/DNA and mammary rating).

The correlation coefficients between the number and weight of placentae and RNA and RNA/DNA were higher when the data of mice with more than 9 placentae each were excluded. This proposes that mammary development, especially its functional state, in mice with 8 placentae would mostly reach its physiological upper limit at late pregnancy.

The lactogenic effect of placental mammotropin hormones appears to be more prominent than their mammogenic effect, because the number and weight of placentae had higher correlations and regression coefficients with RNA and RNA/DNA than with DNA.

Although placental mammotropin may participate mainly in mammary development, placenta is known to secrete estrogen-like and progesterone-like substances as well as mammotropin. Pregnancy is not maintained without ovary (Averill et al., 1950). Dependence of mammogenic and lactogenic effects of placenta on ovarian steroids and adrenocortico-steroids, respectively, has been reported (Ray et al., 1955; Lyons, 1944, 1958; Matthies, 1967). Furthermore, the placental mammotropin was found to have a luteotropic action as well (Averill et al., 1950; Cerruti and Lyons, 1960; Matthies and Lyons, 1971). Therefore, the direct and indirect participation of steroid hormones from placenta, ovary and/or adrenal in the mammary development during pregnancy must also be taken into consideration. Whether placental mammotropin is a principal hormone for mammogenesis as the case of pituitary mammotropin (prolactin) still remains to be solved.

No correlations between the pituitary levels of prolactin of GH and any index of mammary development obtained in the present experiment confirm the view that the wide functional significance of placenta as an adjunct to pituitary is emphasized for the mammary development during pregnancy, especially during the latter half of pregnancy (Amoroso and Porter, 1966).

References


