GONADO-PITUITARY RELATIONSHIPS IN THE FETAL MOUSE AT VARIOUS TIMES DURING SEXUAL DIFFERENTIATION

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SUMMARY

Levels of testosterone in plasma and concentrations of LH in both plasma and pituitary glands of fetal mice aged 14, 16 and 18 days were measured by radioimmunoassays in a representative number of fetuses. During this period levels of testosterone in the plasma of male mice were significantly higher than those in the females. Levels of testosterone in plasma of male mice increased from day 14 to day 16 of gestation and decreased on day 1 before parturition. Plasma concentrations of LH remained undetectable in male and female fetuses until day 16 of gestation. Levels of LH rose slightly in both sexes in later gestation, but still remained significantly lower in the plasma of male fetuses on days 17–18. In contrast, higher but not significantly different concentrations of LH were observed in pituitary glands from days 14 to 18 in male compared with female mice. These observations suggest that the high levels of testosterone in the plasma of male fetal mice might be responsible for feedback inhibition of LH secretion during the last days of gestation.

INTRODUCTION

According to the proposal made by Jost (1947), the development of the male phenotype is dependent upon androgens secreted by the fetal testis. Although it has been shown in experiments in vitro that testosterone secretion by the fetal testes of rodents may be stimulated by exogenous gonadotrophins (Catt, Dufau, Neaves, Walsh & Wilson, 1975; Warren, Halmeyer & Eik-Nes, 1975; Weniger & Zeis, 1975), the relationship between gonadotrophic hormones and testosterone is not clearly understood. Moreover, the origin of these gonadotrophins in vivo is still questionable. It has been suggested that testosterone production by fetal rat testes is under pituitary control (Chowdhury & Steinberger, 1976). Recent studies by Pointis & Mahoudeau (1977) have demonstrated that the pituitary gland of the fetal mouse exhibits a biological gonadotrophin activity during the latter stages of pregnancy. In the present work, specific assays were used to determine whether in-vivo relationships could exist between levels of testosterone and luteinizing hormone (LH) in the plasma of mouse fetuses during sexual differentiation. Preliminary results have been reported previously (Pointis, Mahoudeau & Cedard, 1978).

MATERIALS AND METHODS

Albino Swiss female and male mice were mated for 1 night. Fertilization was verified by the presence of vaginal plugs on the following morning (this day was designated as day 1 of gestation). The average period of gestation in this colony was 19 days. On days 14, 16, 17 and 18 of pregnancy, mice were killed by cervical dislocation and the fetuses with their placentae were removed. To obtain sufficient plasma, fetal blood was collected by decapitation and
pooled into heparinized tubes, after determining the gonadal sex of each fetus by dissection under a stereobinocular microscope. Fetal plasma, obtained following centrifugation, was stored at $-20 \, ^\circ C$ until processed. Subsequently pituitary glands from several male and female fetuses were pooled as follows: day 14, 20 pituitary glands/pool; day 16, 10 pituitary glands/pool; day 18, 5 pituitary glands/pool. The pituitary glands were then homogenized in ice-cold phosphate-buffered saline (PBS) and used for radioimmunoassay of LH.

**Radioimmunoassay of testosterone**

Radioimmunoassay of testosterone was performed on a pool of fetal plasma as indicated in Table 1. The method used for radioimmunological analysis of testosterone has been previously described (Mahoudeau & Briculaire, 1972). Anti-testosterone serum was obtained from rabbits by injection of testosterone-3-0-carboxymethyl-oxime bovine serum albumin. Due to the high cross-reactivity of the antiserum with dihydrotestosterone (90%), radioimmunoassay of testosterone was performed after separation of the plasma on a Celite column. Concentrations of dihydrotestosterone remained undetectable over the observed period. Similar results have been previously reported in experiments *in vitro* (Pointis & Mahoudeau, 1974).

**Table 1. Experimental protocol for the measurement of testosterone in the plasma of fetal mice**

<table>
<thead>
<tr>
<th>Age of fetus (days)</th>
<th>Sex</th>
<th>Lowest and highest number of fetuses from which plasma pooled</th>
<th>No. of pools measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Male</td>
<td>55–132</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>77–121</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>Male</td>
<td>33–73</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>37–63</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>Male</td>
<td>18–40</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>15–33</td>
<td>3</td>
</tr>
</tbody>
</table>

**Radioimmunoassay of LH**

Plasma and pituitary LH contents were measured in duplicate by a double-antibody ovine system using NIAMDD-rat-LH-RP-1 as the standard hormone and an antiserum to ovine LH, as described by Niswender, Midgley, Monroe & Reichert (1968). A representative number of plasma samples collected from orchidectomized adult mice were serially diluted in PBS and assayed to test the adequacy of the cross-reaction of mouse LH with ovine anti-LH antiserum. Data were analysed using a computer program based upon the log-logit linear transformation of the standard curve.

**Statistical analysis**

The significance of differences between two mean values was determined by unpaired *t*-test, with a *P* value of $\leq 0.05$ regarded as significant.

**RESULTS**

**Levels of testosterone in the plasma of fetal mice**

The levels of testosterone in the plasma of male and female fetuses are presented in Fig. 1. In female mice, testosterone concentrations were low and appeared to be unchanged from day 14 to day 18 of gestation. In contrast, the levels of testosterone in male fetuses rose significantly from day 14 to day 16 of gestation ($P < 0.05$), to reach $1140 \pm 166$ (s.e.m.) pg/ml on day 16, before declining on day 18. This level was, nevertheless, higher than that in female fetal animals of the same age ($P < 0.01$) and on day 14 of gestation ($P < 0.01$).
Testosterone and LH levels in fetal mice

Fig. 1. Mean (±S.E.M.) levels of testosterone in the plasma of male (white bars) and female (black bars) fetal mice on days 14, 16 and 18 of gestation.

Levels of LH in the plasma and pituitary gland of fetal mice

Straight lines were obtained when a logit transformation was performed on the ratio of bound: free counts for dilutions of rat LH standard as well as for samples of mouse plasma. The means (±S.E.M.) of the slopes of the two standard curves calculated from ten assay dilutions were not significantly different with NIAMDD-rat-LH-RP-1 (−2.465 ± 0.066) or with dilutions of mouse plasma (−2.563 ± 0.086).

Table 2 shows the levels of LH in plasma of fetal animals. The concentration of immunoreactive LH in the pooled plasma from male and female fetuses remained undetectable on day 14 of gestation and was only detectable by day 16. At this time a sex difference was found, with higher levels in female fetuses. However, the levels of LH in the female fetuses were significantly different from those of the male fetuses only on days 17 (P<0.05) and 18 (P<0.02).

It should be noted that the levels of LH rose in both sexes with increasing fetal age. Significant differences between male and female fetuses were observed between all days,

Table 2. Levels of LH in the plasma of male and female fetal mice from days 14 to 18 of gestation (means ± S.E.M.)

<table>
<thead>
<tr>
<th>Age of fetus (days)</th>
<th>LH (ng/ml plasma)*</th>
<th>Comparison between male and female fetuses (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>14</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>16</td>
<td>8.22 ± 3.60 (4)</td>
<td>17.81 ± 5.13 (5)</td>
</tr>
<tr>
<td>17</td>
<td>13.57 ± 2.97 (3)</td>
<td>27.70 ± 2.66 (3)</td>
</tr>
<tr>
<td>18</td>
<td>22.35 ± 1.83 (4)</td>
<td>76.67 ± 13.25 (3)</td>
</tr>
</tbody>
</table>

* Expressed in ng NIAMDD-rat-LH-RP-1.
ND, Non-detectable levels.
The number in parentheses indicates number of pools measured. Fetal animals from three pregnant mice (average of 15–21) were pooled for each age.
except days 16 and 17; for example there was a 65% rise in the level of LH in male fetuses from day 17 to day 18 ($P<0.05$) and a 177% rise in LH in female fetuses over the same period ($P<0.05$).

Table 3 shows the concentrations of LH in the pituitary glands of fetal mice. Luteinizing hormone was detectable in both sexes at all ages tested. The concentration of LH rose with increasing fetal age in both sexes. Higher levels were found in male fetuses, but the only significant difference between the LH content of male and female pituitary glands was observed on day 14 of gestation ($P<0.02$).

Table 3. LH content of the pituitary glands of male and female fetal mice (means ± s.e.m.)

<table>
<thead>
<tr>
<th>Age of fetus (days)</th>
<th>LH (ng/pituitary gland)*</th>
<th>Comparison between male and female fetuses ($P$ value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>11.84 ± 0.77 (5)</td>
<td>8.04 ± 0.80 (4) &lt; 0.02</td>
</tr>
<tr>
<td>16</td>
<td>33.48 ± 5.62 (4)</td>
<td>21.53 ± 3.25 (3) &gt; 0.05</td>
</tr>
<tr>
<td>18</td>
<td>57.49 ± 3.30 (5)</td>
<td>47.92 ± 3.39 (5) &gt; 0.05</td>
</tr>
</tbody>
</table>

* Expressed in ng NIAMDD-rat-LH-RP-1.

The number in parentheses indicates number of pools measured.

**DISCUSSION**

The present results show that the levels of testosterone in the plasma of male fetuses were raised during differentiation of the genital tract. It is probable that the testosterone detected in fetal blood was of fetal origin, since maternal levels of testosterone are low at all stages of pregnancy (Barkley, Michael, Geschwind & Bradford, 1977). Moreover, the high concentrations of testosterone found in the male fetuses, as compared with the lower levels detected in female fetuses, suggest that this steroid is secreted by the fetal testis. The origin of these detectable levels of testosterone in the circulation of the female fetuses is not clearly understood. Fetal ovaries and/or adrenals may be the possible sources. It is also conceivable that maternal blood, as well as the placenta, may also contribute to the level of testosterone in the female fetuses.

The validity of the radioimmunological analysis used in the present study for measurement of plasma and pituitary LH in mice, is supported by the following observations. Anti-ovine LH antiserum is specific for LH (Niswender et al. 1968). Our results show good parallelism between rat standard curves and those obtained with serial dilutions of mouse plasma, suggesting a total cross-reaction. Finally, a transitory rise in immunoreactive and biologically active LH was observed in culture media from fetal mouse pituitary glands exposed to synthetic gonadotrophin releasing hormone (Pointis & Mahoudeau, 1976).

Our results show that the levels of LH remain below the level of detection in plasma from 14-day-old male fetuses. These findings are in agreement with previous reports, based on studies *in vitro*, regarding the absence of gonadotrophic activity by pituitary tissue of the same age (Pointis & Mahoudeau, 1977), and with earlier experiments in which the removal of the fetal pituitary gland failed to inhibit differentiation of the genital tract (Raynaud & Frilley, 1947). From the results of the above two studies, it is tempting to suggest that the androgenic activity of the fetal testis is not controlled at this time by gonadotrophins of fetal pituitary origin. However, the fact that LH is detectable in the age-matched pituitary glands is difficult to reconcile with this interpretation. Perhaps the fetal pituitary gland only releases small quantities of gonadotrophins which are not detected in both the bioassay and radioimmunoassay systems used. It is also conceivable that the fetal pituitary gland is not capable of releasing gonadotrophic hormones in the absence of gonadotrophin releasing hormone (Gross & Baker, 1977). However, there is compelling evidence that mouse and rat
fetal testes are responsive to gonadotrophins (Weniger & Zeis, 1975; Picon & Ktorza, 1976) and that LH: human chorionic gonadotrophin receptors are present on the Leydig cell membranes of fetal rabbits (Catt et al. 1975). The possibility that gonadotrophins originating in the maternal pituitary gland or placenta may regulate testosterone secretion at this time, should be considered.

The apparent lack of placental transfer of LH from the maternal compartment (Foster, Karsch & Nalbandov, 1972; Rajaniemi & Niemi, 1974) coupled with observations that, in the mouse, LH concentrations in maternal blood are low in the last stage of pregnancy (Murr, Bradford & Geschwind, 1974), may suggest that testosterone secretion by the fetus is not regulated by LH from the maternal pituitary gland. It has been shown that the placenta may be a source of gonadotrophins in the rat and mouse (Haour, Tell & Sanchez, 1976; Wide and Hobson, 1977). However, the actual effect of placental gonadotrophins on the fetal testis is still in doubt. It is also possible that maternal C21 steroids may serve as substrate for testosterone biosynthesis by the fetus. In the mouse, high levels of progesterone are present in both maternal and fetal blood, and can reach the fetal testis to be metabolized (Pointis, Latreille, Mignot, Janssens & Cedard, 1979). Thus it is conceivable that the androgenic function of the fetal testis is regulated by a ‘multifactor control system’ which includes testosterone precursors of maternal and fetal origin, as well as gonadotrophins of placental and/or fetal origin, as suggested by Sanyal & Villec (1977).

In the present work, the existence of close gonado-pituitary relationships in the male fetus during the latter stages of male development are reported. Firstly, the presence of LH in both the blood and pituitary gland as early as day 16 of fetal life suggests that the pituitary gland of the fetal mouse could be participating in the control of testicular activity. These results parallel the ultrastructural studies as well as the immunocytochemical evaluation of gonadotrophic activity in the pituitary gland of fetal rodents (Sano & Sasaki, 1969; Tougard, Picart & Tixier-Vidal, 1977). Secondly, a sex difference in the concentrations of LH in plasma was observed from day 16 to day 18 of fetal development. In contrast to the high levels of LH in the female fetuses in this period, the rise in the levels of LH in the male fetuses was small. Moreover, the increase in LH in male fetuses occurred precisely during the period when testosterone levels in plasma dropped significantly. On the basis of these observations, it seemed reasonable to speculate that the sex difference as regards concentrations of LH results from the marked differences in steroid production by the gonads at this time. The high levels of testosterone in male fetuses could be partly responsible for feedback inhibition of LH release and consequently a higher content of LH in the pituitary gland (Table 3). However, it is not clear from the present study whether the inhibitory role of testosterone is mediated at the level of the pituitary gland, hypothalamus or both. Indirect evidence in support of this negative feedback has come from several studies. The immunization of pregnant rats against testosterone decreases the levels of testosterone in the circulation of male rat fetuses, which in turn increases the in-vitro biosynthesis of testosterone by the testes from the treated fetuses (Goldman, Baker, Chen & Wieland, 1972). These results were explained by the authors on the basis of reflex stimulation of the fetal pituitary-gonadal axis. Similar findings have been reported more recently in the rabbit (Veysiere, Berger, Jean-Faucher, de Turckheim & Jean, 1979). In contrast, the increase in levels of testosterone in the circulation decreases the in-vitro biosynthesis by the fetal rat testis (Chouraqui, Zeis & Weniger, 1977). Together, all these studies strongly suggest an inhibitory role for testosterone in the gonadotrophic activity of the fetal pituitary gland.

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