Electron Microscopic Study on the Fetal Thyroid C Cells Following Fetal Hypophyseoprunia with or without TSH and GH Therapies in the Rat

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(Received for publication May 31, 1980)

Abstract. Thyroid C cells in fetal rats on days 21 and 22 of gestation had well-developed rough endoplasmic reticula (RER) and Golgi complexes and numerous secretory granules. The granules had vascular polarity in their location and some of them were attached to the cell membranes. Fetal hypophyseoprunia by subtotal decapitation (SD) caused fetal hypercalcemia, accompanied by a wide variation in electron density of the secretory granules, an increase in number of flattened RER and a further development of Golgi complexes. These changes in fine structure suggest a rise in calcitonin-producing activity of C cells, probably in response to hypercalcemia. Fetal thyroidectomy caused fetal hypocalcemia, probably owing to parathormone deficiency. TSH therapy of SD fetuses neither prevented the fetal hypercalcemia nor repaired the cytologic changes in C cells. GH therapy of SD fetuses partially prevented the hypercalcemia between day 20 and day 21 of gestation, but not between day 21 and day 22. Cytologic repair was not observed. Propylthiouracil injected into fetuses influenced neither the C cell's structure nor the plasma calcium levels. The observations suggest that the fetal hypercalcemia and the rise in secretory activity of C cells induced by fetal SD are not solely due to a deficiency of fetal pituitary TSH. The role of pituitary GH is questionable. Deficiencies of other brain factors should be considered as contributing to the observations.

Thyroid C cells were formerly called "parafollicular cells" [21] and are now believed to be originated from the ultimobranchial body [1, 26]. C cells are located between follicular cells and basement membrane, and occur singly or in groups [24]. They produce thyrocalcitonin or calcitonin (CT) which plays a role in lowering plasma calcium levels [12, 13, 22, 24]. According to organ culture experiments with fetal rat thyroids, the CT-secretory activity of C cells is augmented as the concentration of calcium in the culture medium is increased [4]. In those culture media which have high concentrations of calcium, secretory granules in C cells are decreased both in number and in electron density, a phenomenon closely paralleled by the increased CT levels in the media [25]. Hypophysectomy of an adult rat is usually said to have no influence on the fine structure of C cells [15, 20]. However, when both

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the plasma calcium level and the secretory activity of C cells are elevated, hypophysectomy can induce hypofunction as shown in fine structure of C cells [15]. In fetal rats, hypophyseoprivia by fetal subtotal decapitation can also induce an elevation of calcium level in fetal plasma, where the level is significantly higher than that in maternal plasma [10, 14]. Jost et al. [14] observed fetal hypercalcemia after the administration of propylthiouracil (PTU) to pregnant rats. If these phenomena are ascribable to the decline of CT-secretory activity of C cells, they should be supported by electron microscopic observation of cytollogic changes in the C cells.

The present study, therefore, was conducted to clarify the degree to which changes would be induced in the fine structure of fetal C cells after fetal subtotal decapitation followed by TSH or GH therapy and after injection of PTU into fetal rats. Fetal plasma calcium levels were also determined.

Materials and Methods

Wistar rats were fed a commercial diet (Oriental pellets NMF) and water. The morning on which mating was detected by the presence of sperm in the vaginal smear was regarded as day 1 of gestation.

On days 20, 21 and 22 of gestation, normal fetuses were obtained from untreated gravid rats in order to determine fetal and maternal plasma calcium and phosphate levels and to observe normal fetal thyroid C cells by electron microscopy.

On day 20 or 21 of gestation, pregnant rats were laparotomized under ether anesthesia, and their fetuses (2 or 3 in a litter) were subjected to surgical total decapitation (TD), subtotal decapitation (SD), thyroidectomy (thyroparathyroidectomy) or injection of PTU. The animals were killed one day later. Fetal blood samples were pooled by each treatment group. Thyroids from fetuses, except those subjected to TD and thyroidectomy, were used for electron microscopic observation.

TD was performed by the method of Domm and Leroy [2]. In this method, a ligature is placed around the fetal neck, and the fetal thyroid is easily damaged. TD results in simultaneous removal of the hypophysis and the thyroid. SD involves ligation through the oral-neck level, leaving the lower jaw and the thyroid intact and removing the hypophysis. Some fetuses subjected to SD were given a subcutaneous injection of 1 IU TSH (Sigma) in 0.1 ml of warm 20% gelatine solution, and some were given an injection of 0.5 mg GH (NIH-Ovine GH or Miles Lab, Bovine Somatotropin) in 0.1 ml of the gelatine solution, at the time of surgery. The injected gelatine solution was soon hardened, and the included hormone would act long by slow absorption by fetal tissues. The animals were killed one day later. Thyroidectomy was performed by the method of Eguchi and Morikawa [3]. PTU injection was made at a dose of 0.5 mg/fetus on day 20 and at a dose of 0.8 mg/fetus on day 21 of gestation (both suspended in 0.1 ml saline); each injected dose corresponded to approximately 0.2 mg/g body weight. Some littermates of PTU-injected fetuses were given saline solution alone.

Plasma phosphate levels in normal fetuses and mothers were determined by the colorimetric method of Fiske and Subbarow [5]. Plasma calcium levels were determined by Baar's nuclear fast red method, as modified by Kingsley and Robnett [16]. For electron microscopic observation, fetal thyroids were quickly removed and fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde (adjusted to pH 7.5 with 0.1 M phosphate buffer) at 0–4°C for 90 min. The glands were rinsed in cold 0.1 M phosphate buffer, postfixed in cold 1% osmium tetroxide with 0.1 M phosphate buffer for 90 min, again rinsed in 0.1 M phosphate buffer, dehydrated in a graded series of ethanol, equilibrated with propylene oxide, and embedded in Epon 812 [19]. Thin sections were made on a Porter-Blum MT-1 ultramicrotome, stained with 2% uranyl acetate in 70% ethanol and lead citrate and were examined with a JEM-100 U electron microscope.

Biological effects of injected TSH and PTU were determined by electron microscopic examination of thyroid follicular cells. Effects of injected GH were determined by light microscopic examination of hematoxylin-eosin-stained sections of Bouin-fixed fetal thyrias.

Results

Plasma calcium and phosphate levels in normal mothers and their fetuses

Maternal levels of both calcium and phosphate declined somewhat during the late period of gestation, near term (Table 1).
Table 1. Changes in calcium and phosphate levels in fetal and maternal plasma during late gestation

<table>
<thead>
<tr>
<th>Gestational age (days)</th>
<th>Plasma calcium (mg/100 ml)</th>
<th>Plasma phosphate (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maternal</td>
<td>Fetal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maternal</td>
</tr>
<tr>
<td>20</td>
<td>7.3±0.7 (7)</td>
<td>10.0±0.9 (7)</td>
</tr>
<tr>
<td>21</td>
<td>7.2±0.5 (8)</td>
<td>8.8±0.6 (8)</td>
</tr>
<tr>
<td>22</td>
<td>6.8±0.7 (7)</td>
<td>9.0±0.4 (7)</td>
</tr>
</tbody>
</table>

Number of animals (maternal) and number of determinations (fetal) are given in parentheses. Fetal plasma was pooled from several fetuses in each litter. Each value is expressed as mean ± S.E.M.

Table 2. Changes in plasma calcium levels (mg/100 ml) in fetal rats following total decapitation, thyroidectomy, subtotal decapitation, with TSH or GH therapy, or injection of propylthiouracil (PTU)

<table>
<thead>
<tr>
<th>Days at Treatment Autopsy</th>
<th>Kind of fetuses</th>
<th>Kind of treatment of fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total decapitation</td>
<td>Thyroidectomy</td>
</tr>
<tr>
<td>20 21 Experimental (E)</td>
<td>6.9±0.6 (5)</td>
<td>7.0±1.0 (5)</td>
</tr>
<tr>
<td>Control (C)</td>
<td>8.5±0.7 (5)</td>
<td>9.0±1.0 (5)</td>
</tr>
<tr>
<td>Saline-given (S)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>E = C</td>
<td>−1.6±0.4*</td>
<td>−1.9±0.3*</td>
</tr>
<tr>
<td>E = S</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>21 22 Experimental</td>
<td>8.8±0.6 (6)</td>
<td>6.2±0.9 (5)</td>
</tr>
<tr>
<td>Control</td>
<td>10.5±0.5 (6)</td>
<td>9.2±0.8 (5)</td>
</tr>
<tr>
<td>Saline-given</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>E = C</td>
<td>−1.7±0.3*</td>
<td>−3.0±0.6*</td>
</tr>
<tr>
<td>E = S</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Number of determinations is given in parentheses. Total decapitation involves hypophysectomy, thyroidectomy and parathyroidectomy. Subtotal decapitation involves hypophysectomy, but leaves the thyroid and parathyroid intact. TSH was injected at 1 IU/fetus. GH was injected at 0.5 mg/fetus. Propylthiouracil (PTU) was injected at 0.5 mg/fetus on day 20 and 0.8 mg/fetus on day 21 of gestation.

Each value is expressed as mean ± S.E.M.

* Statistically significant (p<0.05) with Student t-test for paired samples.

ns: Not significant.

The fetal plasma calcium levels were far higher than the maternal levels, but declined slightly from day 20 to day 21 of gestation. Fetal plasma phosphate levels were also higher than the maternal levels, and rose between day 20 and day 21.

Plasma calcium levels of fetuses in experimental groups.

TD caused a significant decrease of fetal plasma calcium levels as compared with that of intact controls (Table 2). Thyroidectomy also induced a decrease in the fetal plasma calcium levels. Conversely, SD, which left the thyroids intact, elevated the calcium levels, and TSH injected into SD fetuses did not prevent this elevation. GH therapy prevented the elevation in the ex-
perimental period day 20–21, but did not prevent it during the experimental period day 21–22. PTU failed to show any consistent difference in the calcium levels. Saline also did not effect the level of calcium.

Electron microscopic observations

Fetal thyroid C cells were crowded as the groups of cells in contact with follicular cells, especially around the parathyroid.

The C cells located closely to the parathyroid had many diffused secretory granules already on day 20 of gestation (Fig. 1). Each granule was composed of electron-dense materials, enveloped with a limiting membrane (Fig. 2). Rough endoplasmic reticulum (RER) was not so markedly developed. Many ribosomes were distributed in the cytoplasm as polysomes. On day 21 of gestation, secretory granules were increased in number and tended to be located toward the vascular side of the cell. Electron density of granules varied widely, ranging from pale to dark. The RER cisternae were enlarged and the Golgi complexes were also well developed (Figs. 3, 4). Cytologic features on day 22 of gestation were almost the same as on day 21 (Fig. 5).

Near the center of the thyroid, C cells were located sparsely and contained very few secretory granules. The RER was less developed. However, there were many free ribosomes, most of which constituted polysomes (Fig. 6).

In SD fetuses in the experimental period day 20–21, the electron density of secretory granules of the C cells varied from low to high. The RER cisternae were flattened but elongated (Fig. 7). In SD fetuses in the experimental period day 21–22, the C cells contained many secretory granules, an increased number of flattened but elongated RER and well-developed Golgi complexes accompanied by many vacuoles and vesicles (Figs. 8, 9).

The foregoing features of C cells in SD fetuses were not altered by TSH therapy (Fig. 10). The GH therapy also did not influence features of C cells of SD fetuses. In PTU-treated fetuses, the RER of C cells was neither increased in number nor elongated. The C cells had a relatively large amount of secretory granules which were distributed throughout the cytoplasm (Figs. 11, 12). Golgi complexes were occasionally well developed.

Discussion

The foregoing electron microscopic observations suggest that C cells of normal fetuses secrete actively, at least from day 20 of gestation: secretory granules were polarized toward the vascular side and both RER and Golgi complexes were well developed. The findings are in harmony with those of previous workers [1, 26], supporting the view that C cells of rat thyroids secrete CT during late period of fetal life [8, 14].

As hormones which participate in the regulation of plasma calcium level, CT and parathormone (PTH) can be mentioned. Neither hormone can cross the placenta and both are produced by fetuses themselves [6, 7, 9, 17]. The present observations confirm that the calcium level is higher in fetal plasma than in maternal plasma, supporting the view that alterations in the fetal plasma calcium level are not entirely dependent on alterations in the maternal level [10, 11]. The fetal hypercalcemia seems to decline somewhat toward term in contrast with the elevation of the plasma phosphate level. This is in agreement with the observations of Garel and Pic [10], who pointed out that the decline in plasma calcium level and the rise in plasma phosphate level near the end of fetal life may be intimately associated with the rapid
evolution of ossification in bones.

Fetal hypercalcemia is thought to be a result of fetal acidoses [18]. However, the present experiments seem to imply that the fetal plasma calcium level is dependent on hormones produced by the fetal thyroid and parathyroid glands: both TD and thyroparathyroidectomy caused a decline in the fetal plasma calcium level and SD induced a rise in the level. The decline in the calcium level following TD and thyroparathyroidectomy can be easily explained by the disappearance of PTH. The problem is the rise in the calcium level after SD, where the pituitary is removed but the thyroid is left intact. The hormonal interpretation of this problem may be an increased secretion of PTH and/or a decreased secretion of CT. Furthermore, it is supposed that a deficiency of pituitary hormones is responsible for the rise in the fetal plasma calcium level, whether directly or indirectly.

However, according to the present electron microscopic observations, C cells of SD fetuses contained well-developed Golgi complexes and an increased number of flattened but elongated RER. These changes in C cells of SD fetuses suggest a rise in CT-producing activity of C cells in response to hypercalcemia.

The TSH therapy of SD fetuses failed to repair the hyperfunctional changes in C cells, despite the fact that the amount injected was sufficient to stimulate the follicular cells. Therefore, it is unlikely that the changes induced in C cells of SD fetuses are due to endogenous TSH deficiency. Similarly, PTU induced no appreciable changes in C cells despite the fact that the amount injected was sufficient to cause stimulation of the follicular cells by endogenous TSH from the pituitary. It has been reported that long-term treatment of pregnant rats with PTU induces fetal hypercalcemia [14]. Therefore, if a large amount of PTU is given to fetuses, it may cause destruction of the C cells. In any event, the effect of PTU does not seem to be the same as that of SD.

GH can be mentioned as a possible factor among the pituitary hormones which may influence the plasma calcium level. However, the GH therapy of SD fetuses in the present work also failed to correct changes in C cells despite the amount injected being sufficient to increase the length of the hypertrophied cartilage cell columns in the tibial epiphysis. The hypercalcemia appeared to be slightly improved only in the experimental group day 20–21, in that the difference between GH-injected and control fetuses was not significant. However, this effect is of questionable significance, since it was found only in this group of experimental animals, which showed no cytologic repair.

In conclusion, it should be considered that the fetal hypercalcemia induced by fetal SD may result from deficiencies of brain factors and/or pituitary hormones other than TSH and GH. Also, C cells are thought to be not directly regulated by the pituitary gland in fetuses just as in adults [15, 20]. In any event, the view that the pituitary gland has no direct association with CT secretion of C cells but has direct effects on the plasma calcium levels [4, 27] is worthy of consideration, in the light of results obtained in the present work.

Acknowledgment. This work was supported in part by Grant-in-Aid No. 45625 and No. 548067 from the Ministry of Education, Science and Culture of Japan.

References

[1] Calvert, R. (1972). Electron microscopic observations on the contribution of the ultimobranchial bodies to thyroid histogenesis in the


要約

ラット胎仔下垂体除去後および TSH ならびに GH 投与後の甲状腺 C 細胞の電顕的観察：江口保憲（筑波大学薬学部薬剤学教室）、蒼石光孝・森川喜夫（大阪府立大学農学部畜産学教室）——

胎仔 21 日、22 日のラット胎仔甲状腺 C 細胞はよく発達した粗面小胞体とゴルジ装置および多くの分泌顆粒を保有している。半断頭 (SD) による胎仔下垂体除去後、胎仔血中 Ca 濃度が上昇した。C 細胞の多くでは、粗面小胞体は扁平ではあるが長くなり、明らかに平行にたくさんの積み重なっており、ゴルジ装置は、扁平層状のゴルジ精に加えて、たくさんの空胞、小胞をもなっていた。分泌顆粒は電子密度の低いものから高いものまで、たくさん存在していた。これらの微細構造上の変化は、C 細胞のカルシトニン生産能力の増加を示唆している。これは、高 Ca 血症に対する反応であろう。胎仔の甲状腺除去（上皮小体除去も含む）は、低 Ca 血症をひきおこしたが、これはバラトロン欠損のためであろう。SD 胎仔への TSH 投与は、Ca の血中濃度の上昇を阻止しえず、また細胞学的変化も改善しなかった。SD 胎仔への GH 投与は、胎仔 20-21 日の間で Ca 濃度上昇をいくらか阻止したが、21-22 日の間では阻止しなかった。細胞学的変化はいずれの場合にも改善されなかった。これらの観察は、SD 後の胎仔血中 Ca 濃度の上昇と C 細胞の分泌能の増加は、胎仔下垂体 TSH の欠損によるものではないことを示唆する。下垂体 GH の役割は疑問である。他の下垂体因子や脳の因子が、以上の観察結果に関与を生んでいるかも知れない。
Explanation of Figures

Fig. 1. C cells and follicular cells in the thyroid of a 20-day-old normal fetus. On the left side of the figure, there is a follicular lumen into which are protruded the microvilli of the follicular cells. A C cell located in the left bottom contains many secretory granules. The C cell does not face to the follicular lumen. ×15,000.

Fig. 2. Part of a C cell from a 20-day-old normal fetus. The RER cisternae are small and not markedly enlarged. But, secretory granules are already distributed throughout the cytoplasm. Some granules are attached to the cell membrane. ×32,000.

Fig. 3. A C cell found near the parathyroid from a 21-day-old normal fetus. The RER cisternae are enlarged, and the Golgi complex is well developed (arrow). Secretory granules tend to be arranged close to the cell membrane. ×16,000.

Fig. 4. Part of a C cell from a 21-day-old normal fetus, showing the polarity of granule location toward vascular side (bottom). ×40,000.

Fig. 5. Part of a C cell from a normal 22-day-old fetus, showing many secretory granules and many polysomes. Microtubules can be seen here and there. ×32,000.

Fig. 6. A C cell located about in the center of the thyroid gland from a normal 21-day-old fetus. The cell on the right side is a follicular cell. The C cell on the left side contains scanty polysomes with cell organelles which are not well developed. ×26,000.

Fig. 7. A C cell from a 21-day-old SD fetus. SD was done one day earlier (on day 20). The RER cisternae are flattened and elongated (arrow). ×20,000.

Fig. 8. Part of C cells from a 22-day-old SD fetus. SD was done one day earlier (on day 21). These C cells contain many secretory granules and flattened RER which is stacked in parallel arrangement, particularly in the upper cell. ×15,000.

Fig. 9. Part of a C cell from a 22-day-old SD fetus. SD was done one day earlier (on day 21). Secretory granules have various electron densities. Golgi lamellae are accompanied by many vacuoles and vesicles. The RER cisternae are stacked as flattened and elongated sacs (arrow).

Fig. 10. A C cell from a 22-day-old SD fetus given TSH injection. The cell has secretory granules of various densities and elongated RER cisternae. ×26,000.

Fig. 11. C cells from a 22-day-old fetus given PTU one day earlier. The cell has many secretory granules, some of which are attached to the cell membrane (arrows). ×20,000.

Fig. 12. A C cell from a 22-day-old fetus given PTU one day earlier. The cell abounds with secretory granules with not so many RER cisternae. However, some granules are very close to the cell membrane (arrows). ×40,000.