Age-related Changes of the Ultrastructure in the Parathyroid Gland of the Golden Hamster

By

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Summary: We qualitatively and quantitatively investigated the parathyroid glands of golden hamsters aged 6, 12, 18, 24 and 30 months. Percent area of rER in the parathyroid gland in golden hamsters at 24 months of age was significantly higher when compared to 6 and 12 months of age, and the percent area at 30 months of age was significantly higher when compared to 12 months of age, but there were no significant differences between 24 and 30 months of age. Percent area of the Golgi apparatus at 24 and 30 months of age was significantly higher when compared to 6, 12 and 18 months of age. Ultrastructurally, we believe that in the parathyroid gland of the golden hamster, synthesis and release of parathyroid hormone increase gradually from 6 to 24 months of age and are maintained from 24 to 30 months of age.

Introduction

Previous studies on the development of parathyroid gland (PTG) in various mammals have demonstrated ultrastructural changes in the chief cells of fetus, young and adult animals1–3. Emura et al.4 reported the age-re -lated changes in the ultrastructure of PTG in prenatal golden hamsters until 18 months after birth. It has been reported that the lifespan of the golden hamster is two to three years5, but there have been no studies on the precise fate of PTG in golden hamsters after age 18 months. This investigation was therefore undertaken to study the quantitative and qualitative changes to chief cells in the PTG of adult golden hamsters from age 6 to 30 months.

Materials and Methods

Twenty-five male golden Syrian hamsters were classified into 5 age groups of 5 animals each. All animals were kept under automatically controlled conditions of temperature (23±1°C) and humidity (50±10%), with a 12-h light/dark cycle, and standard laboratory diet (CMF; Oriental Yeast Co., Tokyo, Japan) and drinking water were provided ad libitum. Under deep anesthesia with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight), hamsters were sacrificed at 6, 12, 18, 24 and 30 months of age. Experimental protocols of this study followed the Guidelines for Animal Experi -ments at the School of Dentistry, Aichi-Gakuin University. PTG from animals in all groups was dissected, fixed in a mixture of 2.5% glutaraldehyde and 2% OsO4 in Veronal acetate buffer, pH 7.4, for 1 h at 4°C, followed by dehydration in a graded acetone series and embedding in Epon 812. Body weight of all animals was measured after anesthesia. Ultrathin sections were cut using a Porter-Blum MT-1 ultramicrotome (SORVALL, Newtown, U.S.A.), stained with uranyl acetate and lead salts, and were examined under a JEM-1210 electron microscope (JEOL, Tokyo, Japan). In each hamster from all groups, 20 micrographs at a final magnification of 17,000 were taken at random from different regions of the PTG. Tracing paper was placed over the micrographs and...
outlines of the cytoplasm, the Golgi apparatus (Golgi vesicles, Golgi vacuoles and Golgi lamellae), the cisternae of the rough endoplasmic reticulum (rER), lysosomes, large vacuolar bodies and lipid droplets were drawn. Quantitation of the areas of cytoplasm (= total cell volume minus nucleus), cytoplasmic organelles and cytoplasmic inclusions was performed with a CanoScan 9900F scanner (Canon Inc., Tokyo, Japan) interfaced to an eMac PowerPC G4 computer (Apple, Cupertino, U.S.A.). Scans were saved as 8-bit TIFF files and imported into Adobe Photosh op (Adobe Systems, Mountain View, U.S.A.) and NIH Image program (version 1.63, Wayne Rasband at U.S National Institutes of Health, Bethesda, U.S.A.) for analysis. Regions of the Golgi apparatus, rER, lysosomes, lipid droplets and large vacuolar bodies were expressed as a percentage of the cytoplasmic area. In addition, the number of secretory granule profiles per 100 μm² in the cytoplasm was measured.

Statistical Methods

All data are presented as means ± SEM. One-way analysis of variance (ANOVA) was used to detect significant differences among the 5 groups, and then Fisher’s PLSD test was used to determine differences between pairs of means. Levels of 5% were used to establish the significance of differences.

Results

Body weight

Changes in body weight of golden hamsters in each of the 5 groups are shown in Table 1. Mean body weight in the hamster at 24 months of age was significantly less than at 6 months of age, and at 30 months of age, it was significantly less than at all other ages.

Electron microscopic findings of PTG

In the golden hamsters at all ages, the parenchyma of PTG largely consisted of chief cells, which were oval or polygonal in shape and contained abundant free ribosomes and mitochondria (Figs. 1–5). The fine structure of PTG in each group generally corresponded to previous descriptions4,6).

In the hamster PTG, cytoplasm of the chief cells contained well-developed Golgi apparatus consisting of small vesicles and large, dilated vacuoles with increasing age (Figs. 2–5). In the cytoplasm at all ages, rER was well developed, and at 30 months of age, was arranged as curvilinear whirls rather than as parallel arrays and was randomly distributed (Fig. 5). Lysosomes and irregularly shaped heterogeneously dense bodies (lipofuscin-like bodies) gradually increased in number with age (Figs. 2–5). Large vacuolar bodies containing floccular material or vesicles were sometimes observed in the cytoplasm at all ages (Figs. 1–5). Numerous lipid droplets were particularly noted at 6 months of age (Fig. 1). Numerous secretory granules were scattered in the cytoplasm at all ages except 6 months, and after 12 months of age, numerous secretory granules were situated near the plasma membrane (Figs. 3, 4). The tortuous course of the plasma membranes of adjacent chief cells and the enlargement of intercellular spaces containing floccular or fine particulate materials were frequently observed with advancing age (Fig. 5). In the PTG at 30 months of age, two unusual types of cells were observed among the chief cells: water-clear cells, which had numerous vacuoles containing a fine particulate substance and thread-like material (Fig. 6); and oxyphil cells, which were characterized by the presence of tightly packed, rod-shaped mitochondria with parallel lamellar cristae (Fig. 7).

Morphometry of PTG

The results of morphometric measurements are shown in Table 2. Table 2 shows the organellar profiles of PTG among golden hamsters in all age groups. The mean value for rER in the PTG of hamsters was significantly higher at 24 months of age than that at 6 and 12 months of age, and was significantly higher at 30 months of age than that at 12 months of age, but there were no significant differences between 24 and 30 months of age. The mean value for the Golgi apparatus was significantly higher at 24 and 30 months of age than at 6, 12 and 18 months of age. The mean value for lysosomes was markedly increased from 6 to 18 months of age and was significantly higher at 18, 24 and 30 months of age than at 6 months of age and was significantly higher at 18 months of age than at 12 months of age. The mean value for lipid droplets was significantly lower at 30 months of age than at 6 months of age. The mean value for large vacuolar bodies showed no significant differences among animals at any age. The mean value for secretory granules was significantly lower at 6 months of age than at all other ages, was significantly higher at 12 months of age than at all other ages, and was significantly higher at 18 months of age than at 30 months of age.

Table 1. Comparison of body weight between groups.

<table>
<thead>
<tr>
<th>Age</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 m</td>
<td>173.2±9.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>12 m</td>
<td>165.0±7.1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>18 m</td>
<td>164.8±10.5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>24 m</td>
<td>145.0±3.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>30 m</td>
<td>112.6±4.4&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means±SEM. m: month, a:p<0.05 vs. 6-month group, b:p<0.05 vs. 12-month group, c:p<0.05 vs. 18-month group, d:p<0.05 vs. 24-month group, e:p<0.05 vs. 30-month group.
Fig. 1. Parathyroid chief cells in hamsters at 6 months of age. Chief cells contain abundant free ribosomes, mitochondria, cisternae of rough endoplasmic reticulum (ER), Golgi apparatus (G), lysosomes (L), large vacuolar bodies (V), lipid droplets (LD) and secretory granules. Scale bar = 1 μm.

Fig. 2. Parathyroid chief cells in hamster at 12 months of age. Moderately developed Golgi apparatus (G) and cisternae of rough endoplasmic reticulum (ER) are seen. Many secretory granules (arrows) are situated near the plasma membrane. L: lysosome, V: large vacuolar body. Scale bar = 1 μm.
Fig. 3. Parathyroid chief cells in hamster at 18 months of age. Panel A shows well-developed cisternae of rough endoplasmic reticulum (ER) and panel B shows Golgi apparatus (G). L: lysosome, V: large vacuolar body. Scale bar = 1 μm.

Fig. 4. Parathyroid chief cells in hamster at 24 months of age. Well-developed cisternae of rough endoplasmic reticulum (ER) and Golgi apparatus (G) are seen. L: lysosome, V: large vacuolar body. Scale bar = 1 μm.
Discussion

The rER, the site of parathyroid hormone (PTH) synthesis, the Golgi apparatus, where PTH is packaged into secretory granules, which store and transport PTH to the cell surface, and other organelles (lysosomes, lipid droplets and large vacuolar bodies) related to regulating overproduction of PTH are known to be morphological parameters associated with the functional status of chief cells.

In the present study, the main changes in the cytoplasm of the PTG were an increase in the Golgi apparatus and rER with growth. Cytoplasm at 24 and 30 months of age showed a well-developed Golgi apparatus and rER when compared with 6 and 12 months of age. The large size of the Golgi apparatus and rER suggest an increased capacity for synthesis and packaging of PTH.

In addition, numerous chief cells contained numerous prosecretory granules in the Golgi areas. These findings are similar to the observation of increased in functional activity of PTG.

It has been suggested that lysosomes in PTG increase in number with growth, and may function in the regulation of overproduced secretory granules. Our findings are generally consistent with the observations described above.

Emura et al. demonstrated that large vacuolar bodies decrease with age and increase when the secretory activity of PTG is suppressed in response to acute hypercalcemia. One role of the large vacuolar body is the regulation of overproduction of the secretory process in Golgi areas in the suppressed PTG. In the present study, there were no significant differences among the groups with regard to large vacuolar bodies. However, additional study is required in order to clarify the origin and role of large vacuolar bodies in the PTG.

In the cytoplasm from 6 to 30 months of age, changes in lipid droplets showed the opposite trend as activity of the rER and Golgi apparatus. Nunenz et al. noted that lipid droplets may provide the energy required for the synthesis of PTH when synthesis increases. According to Castleman and Roth, lipid droplets that originate from autophagosomes peak in resting chief cells and are reduced in the active state.

In active chief cells, secretory granules tend to increase in number and gather beneath the plasma membrane. In the present study in the groups after 12 months of age, secretory granules situated near the plasma membrane were numerous when compared with those at 6 months of age, and the enlarged intercellular spaces containing material similar to the contents of secretory granules increased when compared to those

Fig. 5. Parathyroid chief cells of hamster at 30 months of age. Many well-developed cisternae of rough endoplasmic reticulum (ER) and Golgi apparatus (G) are randomly distributed. L: lysosome, V: large vacuolar body, LD: lipid droplet. Insert on the left-hand side of this panel shows the tortuous course of plasma membranes of adjacent chief cells, and the enlarged intercellular space containing floccular or fine particulate materials. Insert at the right-hand side of this panel shows curvilinear whirls of the rough endoplasmic reticulum. Scale bar = 1 μm.
at 6 months of age. These findings suggest that secretory granules in the PTG of the golden hamster may be actively discharged by an eccrine-type secretion\(^{16}\). The number of secretory granules does not correlate with the functional condition of the PTG\(^{17}\). In the present study, the number of secretory granules was highest at 12 months of age, and decreased gradually with age. Accordingly, a decrease in secretory granules in the groups after 12 months of age suggests that the intracellular transport and lysosomal digestion are more markedly stimulated than their synthesis.

In this study, water-clear cells and oxyphil cells were seen in the PTG at 30 months of age. Emura et al.\(^{18}\) reported the presence of water-clear cells containing

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**Table 2.** Comparison of organellar profiles on the PTG among groups.

<table>
<thead>
<tr>
<th>Age</th>
<th>G</th>
<th>ER</th>
<th>Ly</th>
<th>LD</th>
<th>V</th>
<th>SG</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 m</td>
<td>3.63±0.35(^{abc})</td>
<td>3.53±0.32(^{d})</td>
<td>0.30±0.02(^{de})</td>
<td>0.33±0.09(^{e})</td>
<td>0.05±0.01</td>
<td>3.49±0.80(^{cde})</td>
</tr>
<tr>
<td>12 m</td>
<td>4.80±0.29(^{abc})</td>
<td>2.82±0.61(^{abc})</td>
<td>0.46±0.01(^{c})</td>
<td>0.17±0.05</td>
<td>0.06±0.01</td>
<td>19.10±0.36(^{cde})</td>
</tr>
<tr>
<td>18 m</td>
<td>6.07±0.49(^{abc})</td>
<td>4.23±0.40</td>
<td>0.81±0.06(^{ab})</td>
<td>0.29±0.12</td>
<td>0.09±0.02</td>
<td>13.38±1.15(^{abc})</td>
</tr>
<tr>
<td>24 m</td>
<td>7.73±0.48(^{abc})</td>
<td>5.53±0.73(^{ab})</td>
<td>0.66±0.06(^{a})</td>
<td>0.15±0.02</td>
<td>0.05±0.01</td>
<td>11.67±0.96(^{ab})</td>
</tr>
<tr>
<td>30 m</td>
<td>7.51±0.53(^{abc})</td>
<td>5.16±0.92(^{b})</td>
<td>0.69±0.20(^{a})</td>
<td>0.11±0.02(^{a})</td>
<td>0.08±0.01</td>
<td>9.98±1.41(^{abc})</td>
</tr>
</tbody>
</table>

Values are means±SEM. m: month; G: the Golgi apparatus; ER: rough endoplasmic reticulum; Ly: lysosome; V: large vacuolar body; LD: lipid droplet. Values are presented as percentage of cytoplasmic areas. Value of secretory granule (SG) is presented as number of profiles per 100 \(\mu\)m\(^2\) in the cytoplasm. \(^{a}\)p<0.05 vs. 6-month group, \(^{b}\)p<0.05 vs. 12-month group, \(^{c}\)p<0.05 vs. 18-month group, \(^{d}\)p<0.05 vs. 24-month group, \(^{e}\)p<0.05 vs. 30-month group.
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numerous vacuoles within the cytoplasm in the PTG of 3- to 15-month-old golden hamsters. To our knowledge, oxyphil cells have not yet been reported in hamster PTG. Oxyphil cells are characterized by numerous mitochondria located in the cytoplasm and believed to derive from chief cells, occurring only in older animals\(^1\) and in older men\(^{20}\). They are not seen consistently and their function is largely unknown, therefore, previous studies have failed to demonstrate this type of cell in the golden hamster PTG.

In conclusion, ultrastructurally, it appears that the synthesis and release of PTH in the PTG of the golden hamsters increase gradually from 6 to 24 months of age, and are maintained from 24 to 30 months of age.

References