Effect of Immobilization on the Ultrastructure of the Golden Hamster Parathyroid Gland

By

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Key Words: Parathyroid gland, Immobilization, DXA, Ultrastructure, Golden hamster

Summary: To investigate the morphological changes of the parathyroid gland of the immobilized hamsters, we studied the ultrastructure of the parathyroid gland of golden hamsters kept in special small cage (Ballman cage II). All hamsters of the control group were kept in one ordinary cage. Each hamster of the isolated group was kept in ordinary cage individually. Each hamster of the immobilized group was kept in Ballman cage II individually. All hamsters were kept for 5 days. On the first and fifth day of the experiment, bone mineral content (BMC) and bone mineral density (BMD) of whole body were measured by dual energy X-ray absorptiometry (DXA). In the control and isolated groups, BMD of the fifth day was significantly increased as compared to that of the first day. In the immobilized group BMC and body weight were significantly decreased. There was no significant difference among 3 groups concerning the mean serum calcium level. Volume density of the cell organelles and inclusions was estimated and compared among 3 groups. Volume density of the lysosomes and large vacuolar bodies of the isolated and immobilized groups was significantly higher than that of the control group. Much more lipid droplets were observed in the immobilized group than the control and isolated groups. No particular differences were observed as to the Golgi complex in the isolated and the immobilized groups compared to the control group. These findings suggest that the cellular activity of the parathyroid gland is suppressed with immobilization.

Many authors have mentioned that immobilization simulates the weightless condition and affects bone metabolism (Lindgren, 1976; Orsatti et al., 1976; Morey et al., 1979; Weinreb et al., 1989; Yamaguchi and Hoshi, 1992). It has been mentioned that prolonged immobilization may induce hypercalcemia (Elias et al., 1992), hypercalciuria (Stewart et al., 1982) or osteoporosis (Elias et al., 1992; Stewart et al., 1982). Although bone resorption is a principal symptom in immobilized animals, the mechanism of resorption and the role of systemic calcium-regulating hormones in these animals are unclear. Effects of immobilization on parathyroid hormone (PTH) secretion are various through authors: unchanged (LeBlanc et al., 1995), decreased (Vaziri et al., 1994), and increased (Lerman et al., 1977). The ultrastructural study of the parathyroid gland of immobilized animals has not been reported except for one article published by Lindgren and Boquist (1976).

Several methods of immobilization have been reported: suspension by harness (Morey et al., 1979; Anzil et al., 1991); tying down all limbs with adhesive tape on a plastic board (Aou et al., 1993; Ma et al., 1993); spinal paraplegia by severing of the lumbar spinal cord (Krempien et al., 1976), plaster cast at pelvis or limb (Burkhart and Jowsey, 1967; Krempien et al., 1976); external bandage of hind leg (Lindgren and Boquist, 1976).

In the present study we employed special small cages to keep animals under immobilized condition. We studied ultrastructural and quantitative changes of the parathyroid glands of golden hamsters after immobilization. We also refer the changes of bone mineral content (BMC) and bone mineral density (BMD) after immobilization using dual energy X-ray absorptiometry (DXA).
Materials and Methods

Ten-week-old male golden hamsters with an average body weight of 110 g were divided into 3 groups of 5 animals each. Animals of the control group were kept together in an ordinary cage measuring 38 x 26 x 18 cm (Water Flushing Unit Cage, Clea Japan) for 5 days. Each animal of the isolated group was kept in an ordinary cage individually for 5 days. Preliminary study showed that animals kept solitary in a cage moved less than the animals of the control group. Each animal of the immobilized group was kept in a small cage measuring 12 x 4.5 x 4 cm (Ballman cage II, Natsume Seisakusho Co., Ltd.) individually for 5 days. Hamsters received solid chow (CE-2, Clea Japan) and tap water during the experiment ad libitum.

The parathyroid glands were removed from all animals under sodium pentobarbital anesthesia. The glands were immersed in a mixture of 2.5% glutaraldehyde and 2% O2O4 in Millonig's buffer at pH 7.4 for 1 hour, dehydrated through increasing concentrations of acetone, and embedded in Epon 812. Thin sections were cut on a Porter-Blum MT-1 ultramicrotome, stained with uranyl acetate and lead salts, and examined with Hitachi H-800 electron microscope. Twenty micrographs (final magnification of x 22,000) were taken from different regions of the parathyroid glands in each animal. The area of cytoplasm, the Golgi complexes, lipid droplets, large vacuolar bodies and lysosomes, and a number of secretory granules were estimated with the aid of an image analyzer (Image measuring system, Finetec).

The serum calcium levels of all animals were measured using a Corning calcium analyzer 940.

On the first day and fifth day of the experiment BMC and BMD of whole body were measured by DXA, using a Toyo Medic QDR type 2000.

All data were presented as the mean ± SEM. Statistical analysis by one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference test was done using StatView J-4.5 (Abacus Concepts, Inc.) on a Macintosh computer.

Results

Serum Calcium Level

The mean serum calcium levels (mg/100 ml) of the 3 groups are shown in table 1. There was no significant deference among 3 groups regarding to the mean serum calcium level.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.27 ± 0.21</td>
</tr>
<tr>
<td>Isolated</td>
<td>8.86 ± 0.15</td>
</tr>
<tr>
<td>Immobilized</td>
<td>9.23 ± 0.19</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Fine Structure of the Parathyroid Glands

The morphology of the parathyroid glands of the control group resembled that of normal hamsters as reported earlier (Emura et al., 1984; Shoumura et al., 1988a, 1988b, 1988c; Isono et al., 1990). The chief cells were oval or polygonal in shape. The plasma membranes of adjacent cells pursued a tortuous course with occasional interdigitations (Figs. 1, 2). The intercellular spaces were generally narrow. Occasional dilation of the intercellular spaces contained floccular or finely particulate materials (Figs. 1, 2). The cytoplasm was scattered diffusely with mitochondria and free ribosomes (Figs. 1, 2). Golgi complexes were relatively well developed and associated with some prosecretory granules (Fig. 2). Cisternae of the granular endoplasmic reticulum were randomly distributed or arranged in parallel arrays. Secretory granules of 150–300 nm in diameter were frequently observed in the cytoplasm and sometimes located in a peripheral position just beneath the plasma membrane (Figs. 1, 2). Large vacuolar bodies 350–750 nm in diameter contained floccular material or vesicles (Fig. 1). Lysosomes and lipid droplets were sometimes seen in the cytoplasm.

In the parathyroid glands of the isolated and immobilized groups, chief cells had more lysosomes and large vacuolar bodies than those of the control group (Fig. 3, 4).

In the chief cells of the parathyroid glands of the immobilized group, more lipid droplets were observed than in those of the other two groups (Fig. 5, 6).

Stereological Analysis of the Parathyroid Gland

The results of the stereological investigations are given in table 2. In the isolated group, the volume density occupied by the lysosomes and large vacuolar bodies was significantly higher (p < 0.05) than that of the control group. In the immobilized group, the volume density occupied by lysosomes, lipid droplets and large vacuolar bodies was significantly higher (p < 0.05) than that of the control group. There was no significant difference among the 3 groups regarding to the mitochondria and Golgi complex. The number of secretory granule
Table 2. Volume densities of the mitochondria (M), Golgi complexes (G), lysosomes (LY), lipid droplets (LD) and large vacuolar bodies (VB), and number of the secretory granules (SG) per 100 \( \mu \text{m}^2 \) of the cytoplasm

<table>
<thead>
<tr>
<th></th>
<th>M (%)</th>
<th>G (%)</th>
<th>LY (%)</th>
<th>LD (%)</th>
<th>VB (%)</th>
<th>SG (n/100 ( \mu \text{m}^2 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.54 ± 0.26</td>
<td>9.28 ± 0.91</td>
<td>0.39 ± 0.04</td>
<td>0.32 ± 0.12</td>
<td>0.26 ± 0.03</td>
<td>4.82 ± 0.72</td>
</tr>
<tr>
<td>Isolated</td>
<td>8.34 ± 0.25</td>
<td>9.13 ± 0.80</td>
<td>0.57 ± 0.05*</td>
<td>0.71 ± 0.26</td>
<td>0.59 ± 0.03*</td>
<td>6.85 ± 0.95</td>
</tr>
<tr>
<td>Immobilized</td>
<td>8.79 ± 0.54</td>
<td>8.38 ± 0.72</td>
<td>0.55 ± 0.03*</td>
<td>2.78 ± 0.47*</td>
<td>0.47 ± 0.02*</td>
<td>7.20 ± 0.89</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*a: p < 0.05 vs Control; b: p < 0.05 vs Isolated.

Table 3. Bone mineral content (BMC) and bone mineral density (BMD) in the whole body, and body weight

<table>
<thead>
<tr>
<th></th>
<th>BMC (g)</th>
<th>BMD (g/cm²)</th>
<th>Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First day</td>
<td>Fifth day</td>
<td>First day</td>
</tr>
<tr>
<td>Control</td>
<td>2.64 ± 0.12</td>
<td>2.85 ± 0.09</td>
<td>0.098 ± 0.001</td>
</tr>
<tr>
<td>Isolated</td>
<td>2.68 ± 0.11</td>
<td>2.79 ± 0.09</td>
<td>0.102 ± 0.002</td>
</tr>
<tr>
<td>Immobilized</td>
<td>2.66 ± 0.12</td>
<td>2.29 ± 0.08*</td>
<td>0.099 ± 0.002</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*a: p < 0.05 vs First day.

was slightly higher in the isolated and immobilized groups than the control.

**Dual Energy X-ray Absorptiometry (DXA) and Body weight**

Table 3 shows the changes of the BMC, BMD and body weight. In the fifth day of the control and isolated groups, BMD was significantly increased (p < 0.05) as compared to that of the first day of the control and isolated groups. In the fifth day of the immobilized group, BMD was not significantly different as compared to that of the first day of the immobilized group. In the fifth day of the control and isolated groups, BMC and body weight were not significantly different as compared to those of the first day. In the fifth day of the immobilized group BMC and body weight were significantly decreased (p < 0.05) as compared to those of the first day.

**Discussion**

The bone loss induced by immobilization or by the decreased gravitational forces of space is well described. Several authors have reported that the BMD and BMC are decreased by immobilization, which were studied using a DXA scanner (Bourrin et al., 1995; Kannus et al., 1996). The present study demonstrated that in the immobilized group the BMC and body weight decreased. These results are same as Bourrin et al. (1995) and Kannus et al. (1996) reported.

The serum calcium level is known to be the main regulator of the parathyroid hormone secretion. Ma et al. (1993) reported that the mean blood calcium level of the rats significantly fell 2 hours after immobilization. In the present study, no changes were detected in the serum calcium level among the groups.

Lindgren and Boquist (1976), without morphometric procedure, reported that no obvious differences were found in the parathyroid morphology between the immobilized and control rats. The present study demonstrated that in the chief cells of the parathyroid glands of the immobilized groups, volume density of the lysosomes, lipid droplets and large vacuolar bodies were significantly higher than that of the control group. No changes were observed as to the secretory granules, Golgi complex and mitochondria among the 3 groups. Emura et al. recently investigated the changes of large vacuolar bodies in the parathyroid glands of hamsters after short-term treatment with calcium (1992, 1994a), prostaglandin E2 (1994b) or progesterone (1995). The results suggested that the large vacuolar bodies increased with acute hypercalcemia and decreased with hypocalcemia induced by progesterone. In the present study increased large vacuolar bodies in the isolated and immobilized groups may reflect sup-
pressed condition of the parathyroid gland.

Several studies have described that the hypoactive chief cells show an increase in lipid droplets (Isono et al., 1980, 1981, 1982, 1983; Emura et al., 1988, 1994b, 1995; Shoumura et al., 1990; Utsumi, 1993). The present study likewise demonstrated the higher volume density occupied by the lipid droplets in the immobilized group than the control. We consider that the ultrastructural changes observed in the immobilized group are induced by suppression of the cellular activity of the parathyroid gland after immobilization. Increased lysosomes and large vacuolar bodies in the isolated group may reflect the suppressed function of the parathyroid gland induced with less movement.

References


30) Utsumi M, Emura S, Hayakawa D, Yamahira T, Terasawa K, Tamada A, Isono H and Shoumura S. Ultrastructure of the parathyroid gland of magnesium-treated Golden ham-


Explanation of Figures

Plate I

Fig. 1. Electron micrograph of the parathyroid gland of the control golden hamster. Golgi complexes (G) are relatively well developed. Few secretory granules (arrowheads) are observed. LY: lysosomes. L: lipid droplet. V: large vacuolar body. × 11,000

Fig. 2. Electron micrograph of the parathyroid gland of the control golden hamster. Golgi complexes (G) are relatively well developed. A secretory granule (arrowhead) is situated close to the plasma membrane. The plasma membranes of adjacent cells pursue a tortuous course with occasional interdigitations (arrows). LY: lysosome. × 22,000
Plate II

Fig. 3. Electron micrograph of the parathyroid gland of the isolated golden hamster. Some secretory granules (arrowheads) are located in the peripheral cytoplasm. Few lipid droplets (L) are observed. G: Golgi complexes. LY: lysosomes. V: large vacuolar body. × 11,000

Fig. 4. Electron micrograph of the parathyroid gland of the isolated golden hamster. Numerous secretory granules (arrowheads) are observed. G: Golgi complexes. LY: lysosome. L: lipid droplets. × 22,000
Plate III

Fig. 5. Electron micrograph of the parathyroid gland of the immobilized golden hamster. Lipid droplets (L) are numerous. ER: cisternae of the granular endoplasmic reticulum. Arrowheads: secretory granules. × 7,400

Fig. 6. Electron micrograph of the parathyroid gland of the immobilized golden hamster. Lipid droplets (L) are numerous. Several secretory granules (arrowheads) are located in the peripheral cytoplasm. G: Golgi complexes. ER: cisternae of the granular endoplasmic reticulum. LY: lysosome. V: large vacuolar bodies. × 22,000