Acute Effects of Caffeine Administration on the Ultrastructure of the Golden Hamster Parathyroid Gland

By

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Summary: This study was conducted to evaluate the acute effects of caffeine on the ultrastructure of the parathyroid glands in golden hamsters. Caffeine was given orally at either 2.5 mg (low dose) or 10 mg (high dose) per 100 g body weight. Caffeine caused a dose dependent decrease of the serum calcium level 2 hours after administration. Transmission electron microscopy of the parathyroid gland revealed that the volume densities occupied by the Golgi complexes and rough endoplasmic reticulum (RER) were found significantly higher 2 hours after receiving high dose of caffeine. Statistical analysis revealed no significant differences regarding to the bone mineral content (BMC) and bone mineral density (BMD).

It is considered that the synthesis of parathyroid hormone is stimulated following caffeine administration.

Materials and Methods

Animals

A total number of 30 adult, female hamsters were used. Hamsters were 3 months old and approximately 160 g and were divided into 2 groups of 15 animals each. In each group 5 animals were served as controls drinking 0.5 ml/100 g body weight distilled water (DW), 5 animals received 2.5 mg/100 g body weight caffeine in 0.5 ml DW and 5 animals received 10 mg/100 g body weight caffeine in 0.5 ml DW orally. The caffeine used was obtained from the Kishida chemical Co. (Osaka, Japan). The amount of caffeine used in the present experiment is equivalent to 340 mg and 1360 mg/70 kg0.75 respectively in humans, when conversion is based on the metabolic body weight (kg0.75), (Kleiber, 1961). The animals of each group containing 15 hamsters were sacrificed 0.5 and 2 hours after receiving caffeine respectively.

Serum Calcium Measurement

The animals were anesthetized with sodium...
pentobarbital and 3 ml samples of cardiac blood were withdrawn into heparinized syringes for analysis. All samples were centrifuged at 3000× for 10 minutes to obtain plasma fractions. The serum calcium concentration was determined using a 940 Corning calcium analyzer.

Transmission Electron Microscopy of Parathyroid
The parathyroid glands were removed and immersed in a mixture of 2.5% glutaraldehyde and 2% osmium tetroxide in Millonig’s buffer at pH 7.4 for 1 hour, dehydrated through ascending concentrations of acetone and embedded in Epon 812. Thin sections were cut on Porter-Blum MT-1 ultramicrotome, mounted on 200 mesh grids, stained with uranyl acetate and lead salts, and examined with a Hitachi H-800 electron microscope at 100 kV. From the parathyroid glands of each animal 20 medium-power (×6000) electron micrographs were taken at random and enlarged at a final magnification of 22000. The total number of micrographs analyzed was 600.

The areas of cytoplasm, cisternae of the RER, mitochondria, Golgi complexes, secretory granules, lysosomes and large vacuolar bodies were determined using an image measuring system (Finetec).

BMC and BMD of the Whole Body
The BMC and BMD of the whole body were measured for each group of animals. The measurements were done by (DXA) Dual Energy X-ray Absorptiometry (Toyo Medic QDR Type 2000) on the same day of experiment after sacrificing.

Statistics
All of variance data were presented as the mean ± SEM. One way analysis of variance data (ANOVA) was used to detect significant differences among the means in each group containing 15 animals. Fisher’s protected least significant difference comparison test was used to determine differences between pairs of mean. Significance was accepted at p < 0.05.

Results

Serum Calcium Level
The mean serum calcium levels (mg/100 ml) of the control and caffeine-treated groups are shown in the Table 1. The serum calcium concentration of hamsters 2 hours after high dose administration of caffeine was significantly decreased when compared with the controls.

BMC and BMD of the Whole Body
Table 1 sets out the mean values for BMC (g) and BMD (g/cm²) of the whole body.

There were no significant differences between the controls and caffeine-treated animals regarding these values.

Fine Structure of the Parathyroid Gland
Table 2 sets out the mean values for volume density of cell components. The description of the ultrastructure of the control hamster’s parathyroid glands given in this paper agrees with previous reports (Isono et al., 1990; Chen et al., 1999), (Fig. 1). Parathyroid glands of the controls and of caffeine-treated hamsters comprised cells of similar shape and electron density of the cytoplasmic matrix. The volume density of the Golgi complexes associated with some prosecretory granules was estimated to be significantly elevated in the high dose caffeine-treated group after 2 hours (Fig. 2). Cisternae of the RER were sometimes arranged in parallel arrays, distributed in the cytoplasm (Fig. 1). The significant increment of RER was seen 2 hours after high dose caffeine administration comparing to the controls (Fig. 2). Secretory granules varied from round to rod-shaped and measured from 150 to 350 nm in diameter (Fig. 1). Large secretory granules, 350–600 nm in diameter, showed lower electron density than the secretory granules. Large

<table>
<thead>
<tr>
<th>PROTOCOL</th>
<th>TIME (h)</th>
<th>CALCIUM</th>
<th>BMC</th>
<th>BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5</td>
<td>11.00 ± 0.20</td>
<td>3.82 ± 0.092</td>
<td>0.127 ± 0.002</td>
</tr>
<tr>
<td>Low dose</td>
<td>0.5</td>
<td>10.66 ± 0.13</td>
<td>3.86 ± 0.080</td>
<td>0.126 ± 0.003</td>
</tr>
<tr>
<td>High dose</td>
<td>0.5</td>
<td>10.84 ± 0.08</td>
<td>3.73 ± 0.129</td>
<td>0.125 ± 0.002</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>11.17 ± 0.20</td>
<td>3.98 ± 0.166</td>
<td>0.125 ± 0.003</td>
</tr>
<tr>
<td>Low dose</td>
<td>2</td>
<td>10.60 ± 0.21</td>
<td>3.92 ± 0.180</td>
<td>0.125 ± 0.002</td>
</tr>
<tr>
<td>High dose</td>
<td>2</td>
<td>10.43 ± 0.20*</td>
<td>3.93 ± 0.097</td>
<td>0.123 ± 0.004</td>
</tr>
</tbody>
</table>

The values are mean ± SEM. *: significantly different from control group (P < 0.05).
Table 2. Volume density (%) of cell components

<table>
<thead>
<tr>
<th>PROTOCOL</th>
<th>TIME (h)</th>
<th>G</th>
<th>RER</th>
<th>SG</th>
<th>SG mem/SG</th>
<th>M</th>
<th>LY</th>
<th>VB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5</td>
<td>5.17 ± 0.43</td>
<td>6.39 ± 0.43</td>
<td>0.42 ± 0.04</td>
<td>38.59 ± 2.48</td>
<td>9.40 ± 0.29</td>
<td>0.72 ± 0.02</td>
<td>0.65 ± 0.08</td>
</tr>
<tr>
<td>Low dose</td>
<td>0.5</td>
<td>6.22 ± 0.23</td>
<td>7.44 ± 0.99</td>
<td>0.42 ± 0.07</td>
<td>40.85 ± 2.91</td>
<td>9.33 ± 0.43</td>
<td>0.67 ± 0.03</td>
<td>0.66 ± 0.06</td>
</tr>
<tr>
<td>High dose</td>
<td>0.5</td>
<td>5.07 ± 0.91</td>
<td>6.70 ± 0.79</td>
<td>0.31 ± 0.04</td>
<td>32.03 ± 3.91</td>
<td>9.30 ± 0.39</td>
<td>0.76 ± 0.58</td>
<td>0.56 ± 0.07</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>5.94 ± 0.28</td>
<td>6.95 ± 0.93</td>
<td>0.31 ± 0.03</td>
<td>40.68 ± 8.58</td>
<td>9.13 ± 0.11</td>
<td>0.58 ± 0.08</td>
<td>0.82 ± 0.15</td>
</tr>
<tr>
<td>Low dose</td>
<td>2</td>
<td>6.89 ± 0.46</td>
<td>7.67 ± 0.61</td>
<td>0.32 ± 0.03</td>
<td>31.25 ± 2.42</td>
<td>9.49 ± 0.32</td>
<td>0.65 ± 0.11</td>
<td>0.77 ± 0.10</td>
</tr>
<tr>
<td>High dose</td>
<td>2</td>
<td>7.27 ± 0.20*</td>
<td>9.25 ± 0.40*</td>
<td>0.41 ± 0.07</td>
<td>35.29 ± 3.69</td>
<td>9.28 ± 0.33</td>
<td>0.91 ± 0.18</td>
<td>0.48 ± 0.07</td>
</tr>
</tbody>
</table>

The volume densities are presented as percentage of cell volume.
G: Golgi complex; RER: rough endoplasmic reticulum; SG: secretory granule; SG mem/SG: Percentage of secretory granules close to the cell membrane per total secretory granules; M: mitochondria; LY: lysosome; VB: large vacuolar body.
The values are mean ± SEM. *: significantly different from control group (P < 0.05).

Vacuolar bodies of 350–750 nm in diameter, were sometimes observed in the cytoplasm. There were no significant differences among the 6 groups concerning the secretory granules and large vacuolar bodies. Transitional forms between large secretory granules and large vacuolar bodies were present. Lysosomes were sometimes seen in the cytoplasm.

Discussion

Caffeine is known to increase urinary loss of calcium. It has been reported that total urine output of water, calcium and magnesium increased in 2 hours, following caffeine ingestion when compared to the control beverage in adult women (Bergman et al., 1990). According to their study, increased urinary losses of calcium and magnesium arise from significantly reduced percent tubular reabsorption of these 2 minerals. In our study a significant decrease of serum calcium level 2 hours after high dose caffeine administration was observed. It could be due to the dose dependent hypercalciuric effect of caffeine (Heaney and Recker, 1982).

It is known that the PT secretory is mainly controlled by serum calcium level via a negative feedback mechanism. Synthesis of polypeptides is in the RER followed by their transport via the vesicles to the Golgi region. Then the secretory proteins are packed into membrane-bounded granules which are released into the extracellular fluid (Wild and Setoguti, 1995).

The ultrastructural indications of parathyroid gland activity include an increase in volume densities occupied by RER, Golgi complexes and mitochondria. All these features can be used as indicators of increased protein synthesis (Shoumura et al., 1990). Isono and Shoumura (1973) reported that following injection of leucine $^3$H most of the radioautographic grains reached the RER and Golgi complexes after 15 and 30 minutes respectively. The grains were seen in the secretory granules and lipofusin-like bodies at 60 minutes, and at 120 minutes were found in intracellular spaces. In our experiments the time duration was long enough to show the significant increase of RER and Golgi complexes after 2 hours of oral dose of caffeine. We think that the PTH synthesis had been in the beginning of it’s pathway when the PT gland fixation procedure was started. This is why the expected increase in percent of the secretory granules close to the cell membrane (shoumura et al., 1989) or the significant decrease in the volume density of vacuolar bodies (Emura et al., 1997) are not found in our results.

Unfortunately, demonstration of PTH concentration level in hamsters using radioimmunoassay methods has not been possible so far.

No significant differences were found regarding the BMC and BMD of caffeine-treated animals compared with controls. There are studies in agreement with our results (Lloyd et al., 1991). Although the lifetime caffeine intake seems to be associated with decreased bone density in older women (Barrett-Connor et al., 1994).

We conclude that short-term oral doses of caffeine results in changes of the morphometric parameters of parathyroid glands. The osteoporotic changes were not seen in this study.

References


Explanation of Figures

Plate I

Fig. 1. Parathyroid chief cells of the control golden hamster. Golgi complexes (G), lysosome (arrow) and large vacuolar bodies (V) are seen. ×22,000
Plate II

Fig. 2. Parathyroid chief cells of the golden hamster 2 hours after the high dose caffeine administration. Well-developed RER (ER) and Golgi complexes (G) associated with many prosecretory granules (arrows) are seen. ×22,000