Comparison of Human Calcitonin Secretion After a 1-Minute Calcium Infusion in Young Normal and in Elderly Subjects

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Synopsis

Human calcitonin (hCT) response to a 1-minute calcium infusion was studied in 6 male and 4 female young normal subjects and in 6 male and 6 female elderly subjects. In the young subjects plasma hCT levels increased significantly (p<0.01) from mean basal value of 82.3±48.7 pg/ml to maximum level of 407.5±198.0 pg/ml in the male, while it increased from 96.3±89.2 to 216.3±89.1 pg/ml (p<0.05) in the female. In the elderly subjects, it increased from 80.3±56.9 pg/ml to maximum level of 229.2±130.6 pg/ml (p<0.05) in the male, while it increased from 109.2±43.7 to 163.3±55.7 pg/ml (p<0.005) in the female.

There was no significant difference in the basal hCT level of these 4 groups. In both the young and the elderly, however, mean increment of hCT level was significantly (p<0.05) higher in the male than in the female of the same-aged group. In both the female and the male subjects, it was significantly (p<0.05) higher in the young than in the elderly of the same sex. A 1-minute infusion of calcium can be used as a reliable provocative test for hCT secretion in human subjects.

Calcium infusion has been used for provocative test for human calcitonin (hCT) secretion, and the procedure was initially 1 to 4 hrs calcium infusion (Strutridge and Kumar, 1968; Tashjian et al., 1970; Jackson et al., 1973; Silva et al., 1974; Deftos, 1974). Recently, a short calcium infusion over 5 to 10 min (Parthemore et al., 1974) and a 1-minute calcium infusion (Rude and Singer, 1977) have been presumed to be a potent stimulus of calcitonin secretion. This rapid calcium administration was performed in patients with medullary calcinoma and their relatives. hCT secretion in response to calcium infusion has been known to have sex differences, namely the response is lower in female than in male subjects from ages 21 to 46 years (Heath and Size-more, 1977). Furthermore, a progressive fall of serum basal hCT level with age has been reported (Samaan et al., 1975; Ber-lyne et al., 1975). Therefore, it was considered worthwhile to investigate the sex and age differences in hCT response to a 1-minute calcium infusion.

Materials and Methods

Subjects

Six male and 4 female normal young subjects from ages 24 to 40 were studied. They were medical staff and were healthy. Six male and 6 female elderly subjects from ages 50 to 79 years, were also studied for comparison. Nine of the elderly were healthy, 2 females asymptomatic senile osteoporosis and 1 female rheumatoid arthritis without steroid treat-
ment. No drugs related to calcium metabolism had been administrated. Each subject was informed of the experiment and written consent was obtained.

Protocol
Calcium (8.5% calcium gluconate solution) was intravenously infused for the period of one min at the dose of 4 mg/kg of body weight. All subjects, fasting at least for the period of 5 hrs, were kept in supine position and the test was done before lunch at noon. Blood samples were obtained at 0, 1, 2, 5, 15 and 30 min, and were immediately chilled. After centrifugation, plasma was immediately separated and stored at -20°C for later analysis of hCT. hCT concentration of all samples was determined within the same assay. Serum calcium and inorganic phosphate were measured by multichannel Technicon autoanalyzer (Gitelman, 1967; Fiske and Subbarow, 1925).

Radioimmunoassay procedure
Anti-hCT antiserum was obtained by immunization of rabbit with synthetic hCT in Freund's adjuvant. hCT analogue ([Asul.7]-hCT) was labeled with 125I by the method of glucose oxidase with lactoperoxidase, yielding a specific activity ranging from 410 to 510 μCi/μg. Gel filtration on Sephadex G-15 showed two peaks and the peptide fraction was used for assay. Assay procedure was based on the double antibody technique. The inhibition dose 50, which is defined as the amount of inhibitor required to reduce specific binding to 50%, was 110 pg in our assay system. Sensitivity was 49 pg/ml and recovery was 99.9±17.4%. Coefficient of variation was 7.95% within assay and 21.9% between assays. Dilution curves of high hCT concentration sera obtained from patients with medullary carcinoma paralleled well with the standard curve. These assay procedures and its evaluation were previously reported in detail (Okada et al., 1978). Statistical analyses were performed by Student’s t test.

Results

Change of hCT
In the young subjects, plasma hCT level increased from 82.3±48.7 (mean±SD) to peaks at 2 or 5 min with mean maximum level of 407.5±198.0 pg/ml (p<0.01) in the male, while it increased from 96.3±89.2 to 216.3±89.1 pg/ml (p<0.05) in the female subjects. In the elderly subjects, plasma hCT level increased from 80.3±56.9 to a mean maximum level of 229.2±130.6 pg/ml.
ml (p<0.05) in the male, while from 109.2 ±43.7 to 163.3±55.7 pg/ml (p<0.005) in the female subjects (Table 1). Basal hCT level was less than 49 pg/ml in 3 of the young male, in 2 of the young female, in 4 of the elderly male and in 1 of the elderly female. There was no significant difference in the basal hCT levels of these 4 groups. Statistical analysis for hCT levels was summarized in Table 1. Mean increments of hCT levels in the young male and female subjects and the elderly male and female subjects were 362.8±197.5, 120 ±79.9, 148.8±123.5 and 54.2±28.4 pg/ml, respectively. The mean increment was significantly higher (p<0.05) in the young male subjects than in the young female and in the elderly male subjects, and it was significantly higher (p<0.05) in the latter two groups than in the elderly female subjects (Fig. 1).

Change of serum calcium and phosphate

Serum calcium levels were summarized in Table 2. There was no significant difference at 1, 2 and 5 min after the infusion between the young and the elderly or the male and the female subjects, but calcium levels at 0, 15 or 30 min were significantly higher in the young male than in the elderly subjects as shown in Table 2. Serum inorganic phosphate level showed statistically no significant change after calcium infusion.

Side effect

A transient flush sensation developed in all subjects but lasted only for the period of one minute. Electrocardiogram was checked but no significant changes was noted.

Table 2. Serum calcium concentration before and after calcium infusion.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Time before and after Ca infusion (min)</th>
<th>Mean maximum level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Young male</td>
<td>5.1</td>
<td>±0.4</td>
</tr>
<tr>
<td>Young female</td>
<td>4.6</td>
<td>±0.4</td>
</tr>
<tr>
<td>Elderly male</td>
<td>4.5**±0.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Elderly female</td>
<td>4.5*±0.4</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Results are given at mean±SD (mEq/l).
Difference from the value in young male in each time period:
* p<0.05,  ** p<0.01,  *** p<0.005.
Discussion

Calcium infusion as a provocative test for hCT secretion has been widely investigated not only in patients with medullary carcinoma but also in normal subjects. These procedures were calcium gluconate 15 mg/kg of body weight for the period of 4 hrs (Tashjian et al., 1970; Jackson et al., 1973; Heath and Sizemore, 1977), calcium chloride 5 mg/kg/hr for the period of 2–4 hrs. (Deftos 1974), calcium gluconate 3 or 10 mg/kg for 1 hr (Sturtridge and Kumar, 1968; Silva et al., 1974). Rude and Singer (1977) reported, however, that 1 min calcium infusion was effective procedure in the diagnosis of medullary carcinoma. The amount of calcium infused was calculated to raise the serum calcium 2 mg/100 ml using 20% of the lean body weight as the volume of distribution of calcium, so the calcium dose of 4 mg/kg as 10% calcium gluconate was infused. Therefore we decided to infuse intravenously calcium at the dose of 4 mg/kg (8.5% calcium gluconate solution: 0.51 ml/kg) over 1 min period. The significantly increased hCT response to this calcium injection observed in the young and elderly subjects studied suggests that this procedure can be used as a reliable provocative test for hCT secretion in human subjects. No side effects but a transient flush sensation developed and lasted for the period of one minute in all subjects studied. When the same dose of calcium was infused over 5 min period to another group of 6 young and 2 elderly male subjects, the flush sensation did not differ from that of one minute infusion in its degree, but it lasted for several minutes. This led us to perform 1 min rapid infusion as a routine provocative test for hCT secretion. Heath III and Sizemore (1977) reported recently that there was a significant difference between male and female subjects not only in mean basal level, but also in responses to both calcium and pentagastrin infusion tests. Our data showed statistically no significant difference in the mean basal concentrations of hCT between male and female or young and elderly subjects, but showed hCT response to rapid calcium infusion to be lower in female than in male, not only in the young but also in the elderly. This is partially compatible with the report of Heath III and Sizemore. There was no significant difference in serum calcium levels at 1, 2 and 5 min of the injection between the young and elderly or between the male and female subjects, though basal calcium level and calcium levels at 15 and 30 min were higher in the young male than in the elderly male and female subjects. This means that the hypercalcemic stimulus for hCT secretion was the same degree for each group studied. Decreased hCT secretion will be one of the causes of osteoporotic change, since the action of calcitonin has been known to inhibit the bone resorption induced by parathyroid hormone (Friedman and Raisz, 1965; Aliapoulios et al., 1966; Gaillard, 1967; Orimo et al., 1969). It is not known yet, however, if the lower hCT response to calcium infusion observed in our studies has any relation to the development of postmenopausal or senile osteoporosis. Further investigation about the effect of sex hormone on hCT secretion will be needed to presume its precise mechanism.

Acknowledgements

We wish to express our appreciation to Mr. S. Tsushima, Mr. N. Nakagawa and Mr. H. Ogawa for technical assistance, and Mrs. M. Moore and Miss E. Kawagoe for help in preparation of the manuscript. We thank Dainippon Pharmaceutical Co., Ltd. for kind supply of calcium gluconate solution.
References
