NOTE

Phospholipid/Ca\(^{2+}\)-dependent Protein Kinase Activity in Human Parathyroid Adenoma

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Abstract

We have examined the activities of phospholipid/Ca\(^{2+}\)-dependent and cyclic AMP-dependent protein kinases of the parathyroid adenomas and the atrophic glands which were resected from three patients with primary hyperparathyroidism. Phospholipid/Ca\(^{2+}\)-dependent protein kinase activity of atrophic parathyroid gland was exclusively present in cytosol fraction (90.7±12.3%). On the other hand, phospholipid/Ca\(^{2+}\)-dependent protein kinase activity of parathyroid adenomas was 66.9±6.4% in cytosol and 33.1±6.4% in membrane fraction, suggesting a translocation of the enzyme from the cytosol to the membranes. Cyclic AMP-dependent protein kinase activity appeared to be higher in parathyroid adenoma than in atrophic parathyroid gland in both cytosol and membrane fractions.

Accumulating evidence has suggested that phospholipid/Ca\(^{2+}\)-dependent protein kinase (protein kinase C) may play an important role in secretory events in endocrine cells (Fearon and Tashjian, 1985). In the parathyroid gland, it was reported that secretion of parathyroid hormone (PTH) would be induced by an increase in cellular cyclic AMP level (Cohn and Elting, 1983). Recently, Brown et al. (1984) and Muff and Fischer (1986) showed stimulation of PTH secretion by TPA, which is known to serve as a direct activator of protein kinase C (Castagna et al., 1982). In the present study, we have compared protein kinase C activities in cytosolic and membrane fractions of parathyroid adenomas with PTH hypersecretion and atrophic parathyroid glands showing repressed secretion in three patients with primary hyperparathyroidism.

Materials and Methods

Case history of subject

Three patients were diagnosed as primary hyperparathyroidism due to parathyroid adenoma. Patients' laboratory data showed hypercalcemia and a high concentration of serum parathyroid hormone (Table 1). Parathyroidectomy was per-
formed. The size of each resected tumor is indicated in Table 1, and three other parathyroid glands were atrophic. These tumors were histologically adenomas.

Table 1. Clinical and laboratory data for subjects

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Serum Ca (mg/dl)</th>
<th>PTH (ng/ml)</th>
<th>Size of the tumor (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>F</td>
<td>11.8</td>
<td>0.6</td>
<td>1.5 × 1.2 × 1.1</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>M</td>
<td>11.7</td>
<td>8.3</td>
<td>3.0 × 2.0 × 1.2</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>F</td>
<td>14.0</td>
<td>2.8</td>
<td>5.0 × 2.5 × 2.1</td>
</tr>
</tbody>
</table>

Materials

$[^32]P$-ATP was purchased from New England Nuclear, DEAE Sephacel was obtained from Pharmacia, phosphatidylserine, 1,2-diolein and histone (Type IIIIS and IIA) were purchased from Sigma, and E64 (N-(N-(L-3-trans-carboxiran-2-carbonyl)-L-leucyl)-agamatine) was donated by Taisho Pharmaceutical Co.

Methods

1) Tissue preparation

Resected tumors and atrophic parathyroid glands were separately homogenized with buffer I (20 mM Tris/HCl, pH 7.5, 2 mM EGTA, 0.25 M sucrose, 2 mM 2-mercaptoethanol, 50 mM E64). The homogenate was centrifuged at 105,000 × g for 60 min, and the resultant supernatant (cytosol fraction) was used for further study. The pellet was rehomogenized with buffer I containing 0.2% NP-40 and centrifuged at 105,000 × g for 60 min to obtain the supernatant (solubilized membrane fraction).

2) DEAE Sephacel chromatography

For protein kinase C assay, an aliquot of cytosol or solubilized membrane fraction was applied to a DEAE column (bed volume; 1 ml) and eluted with 3 ml of 100 mM NaCl in buffer II (20 mM Tris/HCl, pH 7.5, 2 mM EGTA, 2 mM 2-mercaptoethanol, 50 mM E64). The eluted fractions were dialyzed against 1000 ml of buffer III (10 mM Tris/HCl, pH 7.5, 0.1 mM EGTA, 2 mM 2-mercaptoethanol) and used for protein kinase assay.

3) Protein kinase assay

Protein kinase C was assayed by measuring the phosphorylation of histone (Type IIIIS) (Kik-kawa et al., 1982). The reaction mixture (250 µl) contained 20 mM Tris/HCl, pH 7.5, 5 mM magnesium acetate, 0.02% histone (Type IIIIS), 10 µM $[^32]P$-ATP (8.2 × 10^4 cpm/nmol), 10 µg of phosphatidylserine, 0.2 µg of diolein, 0.5 mM CaCl₂, and sample protein. After incubation for 5 min at 30°C, the reaction was stopped by adding 25% trichloroacetic acid. The acid-precipitable materials were collected on a nitrocellulose membrane filter (pore size 0.45 µm). For the cyclic AMP-dependent protein kinase (protein kinase A) assay, the same assay conditions as used for protein kinase C were employed except that 250 pmol of cyclic AMP was substituted for phosphatidylserine, diolein and CaCl₂, and Type IIA of histone was substituted for Type IIIIS. Basal activity which was obtained in the presence of 0.5 mM EGTA instead of phospholipid, diolein, and CaCl₂ (protein kinase C assay) or in the absence of cyclic AMP (protein kinase A assay) was subtracted from the experimental value.

Results

Protein kinase C activity

Cytosolic protein kinase C activity of parathyroid adenoma (238 ± 121 pmol/min/mg protein) tended to be higher than that of atrophic parathyroid glands (145 ± 98 pmol/min/mg protein), while membrane-associated enzyme activity was significantly higher in parathyroid adenomas (116 ± 64 pmol/min/mg protein) than in atrophic parathyroid glands (14 ± 14 pmol/min/mg protein). The percentage of protein kinase C activity of parathyroid adenomas in cytosol fraction (66.9 ± 6.4%) was lower than that of atrophic parathyroid gland (90.7 ± 12.3%) (Table 2). These results indicate that parathyroid adenomas with hypersecretion of PTH have higher membrane-associated protein kinase C activity than atrophic parathyroid glands.

Protein kinase A activity

The cytosolic protein kinase A activity of atrophic parathyroid gland and adenoma was 52 ± 26 and 92 ± 8 pmol/min/mg protein, respectively, whereas membrane-associated activity of atrophic parathyroid gland and adenoma was 90 ± 34 and 756 ± 694 pmol/
Table 2. Protein kinase C activity in parathyroid adenoma and atrophic parathyroid gland

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Adenoma</th>
<th>Atrophic gland</th>
<th>Membrane</th>
<th>Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytosol (pmol/min/mg protein)</td>
<td>Cytosol</td>
<td>Membrane</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>119 (61.3%)</td>
<td>75 (38.7%)</td>
<td>271 (95.4%)</td>
<td>13 (4.6%)</td>
</tr>
<tr>
<td>2.</td>
<td>236 (73.8%)</td>
<td>84 (26.2%)</td>
<td>72 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>3.</td>
<td>360 (65.5%)</td>
<td>190 (34.5%)</td>
<td>92 (76.7%)</td>
<td>28 (23.3%)</td>
</tr>
<tr>
<td>mean±S.D.</td>
<td>238±121 (66.9±6.4%)</td>
<td>116±64* (33.1±6.4%)</td>
<td>145±98 (90.7±12.3%)</td>
<td>14±14 (9.3±12.3%)</td>
</tr>
</tbody>
</table>

a, b, c: p<0.05, in comparison with protein kinase C activity of cytosol or membrane fraction in atrophic parathyroid gland.

Table 3. Protein kinase A activity in parathyroid adenoma and atrophic parathyroid gland

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Adenoma</th>
<th>Atrophic gland</th>
<th>Membrane</th>
<th>Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytosol (pmol/min/mg protein)</td>
<td>Cytosol</td>
<td>Membrane</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>87 (13.1%)</td>
<td>579 (86.9%)</td>
<td>40 (24.4%)</td>
<td>124 (75.6%)</td>
</tr>
<tr>
<td>2.</td>
<td>101 (6.2%)</td>
<td>1521 (93.8%)</td>
<td>35 (38.5%)</td>
<td>56 (61.5%)</td>
</tr>
<tr>
<td>3.</td>
<td>88 (34.5%)</td>
<td>167 (65.5%)</td>
<td>82 (47.7%)</td>
<td>90 (52.3%)</td>
</tr>
<tr>
<td>mean±S.D.</td>
<td>92±8* (17.9±14.8%)</td>
<td>756±694 (82.1±14.8%)</td>
<td>52±26 (36.9±11.7%)</td>
<td>90±34 (63.1±11.7%)</td>
</tr>
</tbody>
</table>

a: p<0.1, comparison with protein kinase A activity of cytosol or membrane fraction in atrophic parathyroid gland.

Discussion

Secretion of PTH has been shown to be mediated by a cyclic AMP-dependent mechanism (Cohn and Elting, 1983). On the other hand, recent studies using dispersed bovine parathyroid cells have demonstrated that TPA, a potent protein kinase C activator, enhanced PTH secretion in the presence of 1.5 mM calcium without changes in cyclic AMP production (Brown et al., 1984; Muff and Fisher, 1986). These investigators suggested an important role of protein kinase C activity in parathyroid gland PTH secretion.

In the present study, the activity of protein kinase C was examined for parathyroid adenoma and atrophic parathyroid gland. Cytosolic protein kinase C activity of parathyroid adenoma undergoing hypersecretion of PTH was lower than that of atrophic parathyroid tissue with repressed secretion, whereas membrane-associated protein kinase C activity was higher in the adenoma than in atrophic tissue. Recently, substantial evidence has shown that stimulation with TPA or other stimulants induces translocation of protein kinase C from cytosol fraction to
membrane fraction in many cell types such as GH4Cl cells (Fearon and Tashjian, 1985), human platelets (Uratsuji et al., 1985) and pancreatic acinar cells (Noguchi et al., 1985). However, no report was available on the translocation of protein kinase C in hormone-producing adenoma cells showing changes in secretion from the patient. The present observation that protein kinase C activity of human parathyroid adenoma in membrane fraction is increased suggests a close association between protein kinase C activity and hypersecretion of PTH. Our data also have shown that protein kinase A activity of resected human parathyroid adenomas appears to be increased, and this is consistent with the finding by Brown and Thatcher (1982) who demonstrated that upon stimulation with dopamine or isoproterenol protein kinase A activity in bovine parathyroid cells was increased with concomitant PTH secretion, but PTH secretory response to lowering of cytosolic Ca\(^{2+}\) concentration was not associated with changes in cytosolic protein kinase A activity.

Further studies with more samples from patients will be required to gain more insight into the relationship of protein kinase C to hypersecretion of PTH in parathyroid adenoma cells.

References


