Neuropeptide Y- and Somatostatin-Like Immunoreactivities in Ganglioneuroma, Ganglioneuroblastoma and Neuroblastoma

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Abstract

Neuropeptide Y (NPY)- and somatostatin (SS)-like immunoreactivities (LI) were investigated in tumor tissues of one ganglioneuroma (GN), 3 ganglioneuroblastomas (GNB) and one neuroblastoma (NB) by radioimmunoassay. NPY-LI was detected from all 5 tumor tissues (16.4-1247 pmol/g wet tissue). Sephadex G-50 column chromatography and reverse phase high performance liquid chromatography (HPLC) revealed that most of the NPY-LI in tumor extracts was eluted in an identical position to synthetic human NPY except one GNB (case 2). In this case, most of the NPY-LI was eluted in a higher molecular weight region than synthetic human NPY in Sephadex G-50 column chromatography and in a more hydrophobic position in HPLC. SS-LI was detected from 4 tumor extracts except one GNB (case 2) (21.3-787 pmol/g wet tissue). Sephadex G-25 column chromatography and reverse phase HPLC revealed that SS-LI in tumor extracts was eluted just after the void volume and then in the same positions as SS-28 and SS-14. These results suggest that NPY, SS-14 and SS-28 exist in tumor tissues of GN, GNB and NB, and most of the NPY-LI in one GNB was a higher molecular and more hydrophobic form of NPY-LI.

Neuropeptide Y (NPY) is a 36 amino acid peptide, which was originally isolated from the porcine brain (Tatemoto, 1982). NPY is known to exist in the central nervous system, sympathetic ganglia, peripheral sympathetic nerves and adrenal medulla in high concentrations (Lundberg et al., 1982; Adrian et al., 1983 b; Allen et al., 1983). High concentrations of NPY in plasma and tumor tissues of phaeochromocytoma and ganglioneuroblastoma had already been reported (Adrian et al., 1983a; Corder et al., 1984; Allen et al., 1987).

Somatostatin (SS) is a 14 amino acid peptide, which was originally isolated from ovine hypothalamus (Brazeau et al., 1973). SS-like immunoreactivity (LI) is widely distributed in the gastrointestinal, pancreatic and brain tissues of many mammalian species. SS-LI is known to exist in the
adrenal medulla and tumor tissue of phaeochromocytoma (Lundberg et al., 1979; Corder and Lowry 1982). A 28 amino acid peptide (somatostatin-28) was isolated from porcine intestine (Pradayrol et al., 1980) and hypothalamus (Schally et al., 1980), and thought to be a precursor of SS-14.

The co-existence of NPY and SS in rat and human cerebral cortex and rat hypothalamus was known (Cronwall et al., 1984) and high concentrations of NPY-LI in the tumor tissue of somatostatinoma were also reported (Allen et al., 1987).

In this study, we investigated NPY-LI and SS-LI in tumor tissues of GN, GNB and NB by radioimmunoassay with gel column chromatography and reverse phase high performance liquid chromatography (HPLC).

**Materials and Methods**

**Tumor tissue**

Five tumor tissues (one GN, 3 GNB and one NB) were obtained at surgery and stored at -80°C until extracted. As controls, 5 adrenal cortical tumors (3 aldosterone-producing adenomas, one non-functioning adenoma and one cortisol-producing carcinoma) were also obtained at surgery. Tumor tissues were boiled in 1M acetic acid for 10 minutes, homogenized and extracted following the procedure previously reported (Mouri et al., 1989). Tumor extracts were reconstituted in assay buffer (0.1M phosphate buffer, pH7.4 containing 0.1% human serum albumin, 0.2% Triton X-100 and 0.1% sodium azide) and subjected to radioimmunoassay.

**Radioimmunoassay**

NPY-LI and SS-LI in tumor tissue extracts were measured by radioimmunoassay.

The radioimmunoassay of NPY was previously reported (Takahashi et al., 1987 and 1988). The assay of NPY showed 20.7% cross reaction with peptide YY, but less than 0.001% with human pancreatic polypeptide, SS-14 and SS-28.

The radioimmunoassay of SS is as follows. The antibody to SS was raised in a rabbit. SS-14 (Peptide Institute, Minoh-shi, Japan) was conjugated to bovine serum albumin (Sigma Chemical Co., USA) with glutaraldehyde. Conjugated SS was injected into a rabbit with complete Freund's adjuvant. Synthetic SS-14 (Peptide Institute) was used as the standard and tyrosyl SS (Peptide Institute) was used for iodination. 125I-SS was prepared by the modification of the chloramine-T method (Hunter and Greenwood, 1962) without the addition of sodium metabisulfite. The antibody was used in an assay at a final dilution of 1:10000. The radioimmunoassay showed a 100% cross reaction with SS-28 (Peninsula Laboratories, USA) and less than 0.001% with human growth hormone releasing hormone, human corticotropin-releasing hormone, human neuropeptide Y, peptide YY, human pancreatic polypeptide, porcine vasoactive intestinal polypeptide, porcine secretin, human glucagon, substance P and angiotensin I.

The sensitivity of the assay was 1.5±0.4 fmol/tube (n=4, mean±SD). Intra- and interassay coefficients of variation were 9.4% (n=10) and 9.3% (n=6), respectively.

**Chromatography**

The fractionations of NPY-LI and SS-LI in tumor extracts were performed by Sephadex G-50 (superfine, 0.9×56 cm) column chromatography, Sephadex G-25 (superfine, 0.9×26 cm) column chromatography and reverse HPLC with a Waters μ Bondapak C18, 3.9×300 mm column. In Sephadex G-50 column chromatography, the eluent was 4M acetic acid containing 0.2% bovine serum albumin and from each fraction tube 0.6 ml of the eluate was obtained. In Sephadex G-25 column chromatography, the eluent was 1M acetic acid containing 0.5% bovine serum albumin and each fraction collected 0.8 ml of the eluate. In HPLC, a linear gradient from 30–60% solvent B (solvent A=0.1% trifluoracetic acid/water; solvent B=0.1% trifluoroacetic acid/acetonitrile) was performed at a flow rate of 1 ml/min. One ml fractions were collected for radioimmunoassay.

**Statistics**

Statistical analysis was performed by analysis of variance and linear regression analysis was carried out by the least-squares method.
Results

NPY-LI in 5 tumor tissues of GN, GNB and NB ranged from 16.4 to 1247 pmol/g wet tissue (590±494 pmol/g wet tissue, mean±SD) (Table 1), which were significantly higher than those in 5 adrenal cortical tumors (6.75±4.67 pmol/g wet tissue) (p<0.01). Serial twofold dilution curves of all these neuroblastic tumor ex-

Table 1. Neuropeptide Y-like and somatostatin-like immunoreactivities in 5 tumor extracts.

<table>
<thead>
<tr>
<th>Histology</th>
<th>NPY-LI (pmol/g wet tissue)</th>
<th>SS-LI (pmol/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 GN</td>
<td>33.9</td>
<td>21.3</td>
</tr>
<tr>
<td>2 GNB</td>
<td>16.4</td>
<td>n.d.</td>
</tr>
<tr>
<td>3 GNB</td>
<td>688</td>
<td>58.6</td>
</tr>
<tr>
<td>4 GNB</td>
<td>1247</td>
<td>787</td>
</tr>
<tr>
<td>5 NB</td>
<td>966</td>
<td>474</td>
</tr>
</tbody>
</table>

GN: ganglioneuroma, GNB: ganglioneuroblastoma, NB: neuroblastoma. n.d.: not detectable, <1.2 pmol/g wet tissue.

Fig. 1. A typical standard curve of neuropeptide Y (●—●) and typical serial twofold dilution curves or tumor extracts (case 2: △—△, case 4: ○—○). B: counts bound in the presence of labeled and unlabeled ligand, B0: counts bound in the presence of labeled ligand alone.

SS-IL was detected from tumor tissues of one GN, 2 GNB and one NB (21.3±787 pmol/g wet tissue) but not detected from one GNB (<1.2 pmol/g wet tissue) (Table 1). Serial twofold dilution curves of these tumor extracts were parallel with a standard curve of SS-14 (Fig. 2). Tumor tissue levels of SS-LI in GN, GNB and NB were significantly higher than those in adrenal cortical tumors (P<0.025). Low levels of SS-LI were detected from two aldosterone-producing adrenal cortical adenomas (1.68 and 6.53 pmol/g wet tissue), but not detected from the other 3 adrenal cortical tumors (<1.2 pmol/g wet tissue).

Sephadex G-50 column chromatography and reverse phase HPLC revealed that most of the NPY-LI in tumor extracts of GN, GNB and NB was eluted in an identical position to synthetic human NPY except one GNB (case 2). In case 2, most of the NPY-LI was eluted in a higher molecular weight region in Sephadex G-50 column

Fig. 2. A typical standard curve of somatostatin-14 (●—●) and a typical serial twofold dilution curve of a tumor extract (case 4: ○—○).
chromatography and in a more hydrophobic position in reverse phase HPLC (Fig. 3 and 4).

By Sephadex G-25 column chromatography, most of the NPY-LI in tumor extracts was eluted in an identical position to synthetic human NPY, which was after the void volume and before the position of SS-28 (the data are not shown in the Fig.).

Sephadex G-25 column chromatography and reverse phase HPLC revealed that SS-LI in tumor extracts was eluted after the void volume and then in the same positions as SS-28 and SS-14 (Fig. 5 and 6). Sephadex G-50 column chromatography of SS-LI in tumor tissue showed a broad peak corresponding to SS-28 and SS-14 (the data are not shown in Fig.).


Fig. 4. Reverse phase HPLC of neuropeptide Y-LI in tumor extracts. a) a typical elution pattern of NPY-LI in tumor tissues (case 4), b) an elution pattern of NPY-LI in case 2. PYY: the elution position of peptide YY, Met 17(O)NPY: that of NPY with oxidated methionine, NPY: that of synthetic human NPY, S-14: that of SS-14, S-28: that of SS-28.
A significant positive co-relation was found between NPY-LI and SS-LI in 5 tumor tissues of GN, GNB and NB ($r=0.92$, $P<0.025$).

Discussion

In the present study, NPY-LI and SS-LI were detected in the tumor tissues of GN, GNB and NB. Gel column chromatography and HPLC of NPY-LI in tumor extracts showed that most of the NPY-LI in these tumors was eluted in an identical position to synthetic human NPY except in case 2 where most of the NPY-LI was a higher molecular and more hydrophobic form of NPY-LI. Minth et al. (1984) reported the sequence of the cDNA encoding NPY in a phaeochromocytoma and suggested the presence of a NPY precursor. Further studies are needed to determine whether this higher molecular and more hydrophobic form of NPY-LI is a precursor of NPY.

Sephadex G-25 column chromatography and reverse phase HPLC revealed that SS-LI in these tumor extracts was eluted just after the void volume but before the position of SS-28, and then in 2 positions of SS-28 and SS-14. These findings suggest that tumor tissues of GN, GNB and NB also contain SS-14-like and SS-28-like materials. The chromatographical pattern of SS-LI in tumor tissues of GN, GNB and NB is similar to that in phaeochromocytoma (Corder and Lowry 1984).

There was a significant co-relation between NPY-LI and SS-LI in 5 tumor tissues of GN, GNB and NB. High concentrations of tissue NPY in somatostatinoma were reported (Allen et al., 1987). The co-existence of NPY and SS in rat and human cerebral cortex and rat hypothalamus is known (Chronwall et al., 1984). These reports and our results suggest a close relationship between NPY and SS in these tumors.

The pathophysiological roles of NPY and SS in GN, GNB and NB are not clear. NB, GNB and NB sometimes secrete vasoactive intestinal polypeptide (VIP) (Fausa et al., 1973; Said et al., 1975; Tiedemann et al., 1981) and show a clinical picture of WDHA syndrome. One case of VIP-secreting neuroblastoma with intractable diarrhea was reported to be treated with intravenous administration of SS (Tiedemann et al., 1981). In this case, SS infusion
reduced serum VIP, although the clinical symptom of watery diarrhea did not improve. Endogenous SS also may have some influence on the secretion of VIP from the tumor. But further studies are also needed to clarify the pathophysiological roles of SS and/or NPY in GN, GNB and NB.

Acknowledgments

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