Somatostatin-like polypeptides in the plasma and tissues of a patient with a somatostatin-producing tumour

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The existence of multiple molecular forms of the polypeptide hormones and probably of all secretory proteins is a consequence of their mode of biosynthesis. Polypeptides larger than somatostatin (mol.wt. 1600) that possess somatostatin-like immunoreactivity have been detected in extracts of hypothalamus, extrahypothalamic brain, stomach, pancreas and gut. Analysis of newly synthesized proteins in pancreatic islets of the anglerfish (Nocc et al., 1979) and rat (Patzelt et al., 1979) has indicated a mol.wt. of approx. 12 000 for prosomatostatin. Release into the circulation of multiple forms of a hormone is a characteristic of endocrine neoplasms and has been extensively documented in the case of islet tumours. The clinical and morphological features of a patient with a somatostatin-producing tumour of the pancreas and multiple liver metastases has recently been described (Krejs et al., 1979). In the present study, the distribution of molecular forms of the somatostatin-like polypeptides in extracts of the primary tumour of this patient are compared with those released by the primary tumour and by the metastases into plasma.

Blood samples, collected from a peripheral vein by the method of Harris et al. (1978), were taken from the patient in the fasted state and after a 4.2MJ (1000 calorie) mixed meal. Further samples were taken, in the fasted state, after resection of the primary tumour. Tissue was extracted by the method of Conlon et al. (1978a). Plasma samples and tissue extracts were chromatographed on columns of Biogel P-10 and Sephadex G-200 and somatostatin-like polypeptides in the effluent fractions were detected by radioimmunoassay (Harris et al., 1978). Samples were diluted at least 20-fold before assay so that degradation of the radio-labelled tracer and non-specific interference by plasma proteins in the assay were negligible. Amounts of immunoreactivity equivalent to 9–25ng of somatostatin/ml were measured in plasma samples in the fasted state (value in normal subjects <100pg/ml) and increased 8-fold after a mixed meal. Three samples of tissue from the primary tumour contained immunoreactivity equivalent to 0.8, 1.2 and 4.6µg of somatostatin/mg wet wt. of tissue (the normal pancreas content was 31–640pg/mg wet wt. of tissue; McIntosh et al., 1978).

Somatostatin-like polypeptides in plasma samples and tissue extracts were resolved by gel filtration into three main peaks in the 10 000–15 000, 2500–3500 and 1500–2000 mol.wt. zones. The relative amounts of these components were estimated from the area under the peaks and are shown in Table 1. Plasma samples, but not tissue extracts, contained low amounts (<1% of total immunoreactivity) of somatostatin-like immunoreactive material, which was eluted from Sephadex G-200 columns in the 15 000–200 000 mol.wt. zone and may represent smaller immunoreactive peptides bound non-covalently to plasma proteins (Conlon et al., 1978b). The amounts of the higher molecular-weight components relative to the somatostatin-sized component are higher in plasma than in tissue extracts, which probably reflects a lower rate of clearance of the larger peptides from the circulation. The neoplasms responded to a nutrient stimulus with an increased release of all three components, but the rate of release of the 1500–2000-mol.wt. component was much greater than that of the larger peptides. The marked increase in the relative amounts of the 10 000–15 000- and 2500–3500-mol.wt. polypeptides in the post-operative plasma is evidence for an increased rate of release of these components from the metastases compared with the primary tumour. Somatostatin-like polypeptides of mol.wts. approx. 12 000, 3000 and 1600 have been identified in extracts of normal dog pancreas (Conlon et al., 1978a), but only the 1600-mol.wt. component was detected in dog plasma and in the plasma-free effluent of the isolated dog pancreas (Conlon et al., 1978b).

Proteolytic cleavage at sites of pairs of basic amino acids represents the predominant, but not exclusive, means of processing of prohormones. In the light of evidence from biosynthetic studies, it is proposed that the component with mol.wt. approx. 12 000 in normal and tumour tissue represents prosomatostatin, which is specifically cleaved to the approx. 1600-mol.wt. component. The approx. 3000-mol.wt. polypeptide may be a product of non-specific cleavage of the prohormone. In normal pancreatic tissue, the rate of processing of prosomatostatin is rapid so that concentrations of the prohormone and the approx. 3000-mol.wt. component are low and only the 1600-mol.wt. component is released into plasma. In the tumours, however, the rate of conversion of the prohormone into hormone is much slower, so that high concentrations of prosomatostatin and its non-specific cleavage products accumulate in the cells and are subsequently released into plasma. The rate of processing of prosomatostatin appears to be less in the metastases than in the primary tumour.

Table 1. Distribution of the molecular forms of the somatostatin-like polypeptides in tissues and plasma

<table>
<thead>
<tr>
<th>Sample</th>
<th>Approximate molecular weight of the somatostatin-like polypeptides</th>
<th>Immunoreactivity (fraction of total in column effluent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12000</td>
</tr>
<tr>
<td>Tissue extracts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal dog pancreas</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Primary tumour</td>
<td>0.06</td>
<td>0.19</td>
</tr>
<tr>
<td>Somatostatinoma plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-operative, fasted</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Pre-operative, post prandial</td>
<td>0.15</td>
<td>0.18</td>
</tr>
<tr>
<td>Post-operative, fasted</td>
<td>0.45</td>
<td>0.20</td>
</tr>
</tbody>
</table>

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Circulating forms of glucagon and related peptides in normal subjects and uraemic patients

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Radioimmunoassays in combination with gel filtration have been widely used (Weir, 1977; Valverde, 1977; Jaspan & Rubenstein, 1977) to study glucagon-like immunoreactivity in human plasma and multiple molecular species have been encountered. These include materials with mol.wts. 3500, 4500, and >40000, in addition to possible fragments with mol.wt. <2000. In these studies, however, there is inordinate reliance on antibodies that bind only to a limited C-terminal region of glucagon. Radioimmunoassays with these antibodies would not detect large gut glucagon-like immunopeptides (Murphy et al., 1973) or other precursors of glucagon in which the C-terminal region of the hormone is masked while a structure that binds antibodies specific for the N-terminal-to-central region is exposed (Murphy, 1979). In these laboratories, studies on circulating glucagon-like immunopeptides have been carried out with both types of antibody. Also, before gel filtration and radioimmunoassay, the glucagon and related materials were purified by immunoaffinity chromatography with each type of antibody immobilized on separate columns, to avoid interference by plasma constituents.

Diglycidyl ether was used to immobilize the antibodies on Sepharose 4B (Murphy et al., 1977). Plasma samples (20ml) were applied first to columns containing the C-terminal antibodies, equilibrated with 0.04M-NaH₂PO₄/Na₂HPO₄ buffer, containing 0.14M-NaCl. The effluent was directed through the column with N-terminal-to-central antibodies. After separate irrigation of the columns with aqueous NH₃, pH 10.5, to release non-biospecifically bound substances, final effluents were eluted with aqueous trifluoroacetic acid, pH 1.5, and freeze-dried. These were then subjected to gel filtration on Sephadex G-200, in agreement with other observations (Weir, 1977). The latter had apparent mol.wts. 160000 and 68000, in the tissue(s) of origin or on the parent molecules that give rise to the C-terminal extension from the pancreas (Tager & Steiner, 1973). An extension at the C-terminal end of glucagon could occlude the C-terminal immunogenic site. The action of plasma proteinases in unmasking C-terminal immunoreactivity of large glucagon-like immunopeptides has been encountered previously (Murphy, 1979). Even though the release of the material with mol.wt. 4500 is apparently stimulated by arginine, it is still not possible to speculate on whether it is released from the pancreas or gut or whether it is formed in the circulation from a larger precursor. Also, the material may possess biological activity since the exposed N-terminal-to-central region of glucagon is a full agonist of the hormone (Wright et al., 1978).

When materials from plasma of normal fasting subjects were examined, the glucagon-like immunopeptides recovered from the C-terminal column were detected by both types of antibody in a fraction with apparent mol.wt. 3500, comprising about 30% of the effluent glucagon-like immunopeptides and at the void volume. The latter had apparent mol.wts. 160000 and 68000, corresponding to immunoglobulin G and albumin respectively on Sephadex G-200, in agreement with other observations (Weir, 1977). Only one fraction of glucagon-like immunopeptide with mol.wt. 4500 was recovered from the N-terminal-to-central column. This reacted only with the N-terminal-to-central antibodies in radioimmunoassay and accounted for about 10% of the total in plasma. The material with mol.wt. 35000 is probably glucagon, since an approximately 2-fold increase in peak height was observed when fasted subjects received intravenous arginine. Unexpectedly, however, the material with mol.wt. 4500 showed a similar response. The glucagon-like immunopeptides associated with plasma proteins were released by treatment with 0.1% sodium dodecyl sulphate and emerged from Sephadex columns as an apparent mixture of materials with mol.wts. 4500 and 3500.

When the material reacting predominantly with the N-terminal-to-central antibodies was returned to plasma that had been cleared of immunoreactivity, incubated for 20 min at 20°C and repurified, material of the same size (mol.wt. 3500) and immunoreactivity (binding both antibodies equally) as glucagon was obtained. This suggests that the parent material may be a precursor of glucagon similar to that with the C-terminal extension from the pancreas (Tager & Steiner, 1973). An extension at the C-terminal end of glucagon could occlude the C-terminal immunogenic site. The action of plasma proteinases in unmasking C-terminal immunoreactivity of large glucagon-like immunopeptides has been encountered previously (Murphy, 1979). Even though the release of the material with mol.wt. 4500 is apparently stimulated by arginine, it is still not possible to speculate on whether it is released from the pancreas or gut or whether it is formed in the circulation from a larger precursor. Also, the material may possess biological activity since the exposed N-terminal-to-central region of glucagon is a full agonist of the hormone (Wright et al., 1978).

Amounts of immunoreactivity measured with both antibodies in plasma from patients with chronic renal failure (400-600pg/ml) were about three times higher than from normal subjects (100-200pg/ml). When the glucagon-like immunopeptides from the uraemic patients was analysed all the immunoreactivity recovered from the C-terminal column reacted with both antibodies in radioimmunoassay. About 47% had the same size as glucagon, whereas 4% appeared with the plasma proteins. Additional fractions, however, with mol.wts. 7000 and 12500 accounted for about 25% each. Materials recovered from the N-terminal-to-central-region columns accounted for about 8% of the total plasma immunoreactivity and reacted only with the N-terminal-to-central antibodies. Over 90% of this immunoreactivity was associated with the plasma proteins and the remainder had mol.wt. 4500.

Clearly, glucagon accounts for only part of the increased concentrations of glucagon-like immunopeptides in the plasma of uraemic patients. Jaspan & Rubenstein (1977) found an even lower proportion as glucagon than observed in the present paper, but also showed this could be increased by arginine stimulation. It seems likely that glucagon is still degraded outside the kidney in uraemic patients, but at a slower rate than in the kidney of normal subjects. As is the case with normal subjects it is not yet possible to comment with any confidence on the tissue(s) of origin or on the parent molecules that give rise to glucagon-like immunopeptide materials other than glucagon in uraemic patients.

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