Concomitant Production of β-Endorphin in Ectopic ACTH/β-LPH-Producing Tumors

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Abstract

It is generally accepted that ectopic ACTH-producing tumors produce not only ACTH but β-MSH or β-LPH as well. Using a sensitive radioimmunoassay for βh-endorphin, we have demonstrated the presence of β-endorphin immunoreactivity with size heterogeneity according to Sephadex gel chromatography and sodium dodecyl sulfate polyacrylamide gel electrophoresis in 6 ectopic ACTH/β-LPH-producing tumors, providing further evidence for the role of a common precursor to ACTH and β-LPH/β-endorphin in the peptide biosynthesis in these tumors, in a manner similar to the biosynthetic events in the pituitary gland of several species.

It was well known that nonpituitary neoplasms associated with Cushing's syndrome produced not only ACTH but β-MSH as well (Abe et al., 1967; Liddle et al., 1969). Later observation, however, revealed that human β-MSH seems to be an artifact during extraction procedures and that ectopic ACTH-producing tumor and human pituitary gland mainly elaborate β-lipotropin (β-LPH)-like peptide but not β-MSH (Bloomfield et al., 1974; Scott and Lowry, 1974; Gilkes et al., 1975; Hirata et al., 1976a; Bachelot et al., 1977).

Recently, highest concentrations of β-endorphin, a newly isolated morphinomimetic peptide composed of C-terminal fragment (61–91) of β-LPH, were demonstrated in the pituitary glands of several species (Bloom et al., 1977; Krieger et al., 1977; Rossier et al., 1977; Matsukura et al., 1978) along with a large amount of β-LPH (Krieger et al., 1977; Rossier et al., 1977; Pelletier et al., 1977). Furthermore, Mains et al. (1977) and Roberts and Herbert (1977) reported the presence of a common precursor with a large molecular weight in the mouse pituitary tumor cell line (At T-20/D-16v), from which ACTH β-LPH, β-endorphin and other related peptides were shown to be derived. The pituitary gland and ectopic ACTH/β-LPH-producing tumor in man, on the other hand, were shown to contain big forms of ACTH besides the authentic ACTH (Yalow and Berson, 1971; Gewirtz and Yalow, 1974; Hirata et al., 1975a; Lowry et al., 1976; Hirata et al., 1976b). Very recently, concomitant production of ACTH, β- and γ-LPH and β- and α-endorphin has been observed in the nonpituitary neoplasms (Orth et al., 1978; Bertagna et al., 1978), suggesting that the biosynthetic pathway of the peptides in the tumors may be quite similar to that in the mouse pituitary tumor (Mains et al., 1977; Roberts and Herbert, 1977).

The present study was undertaken to clarify the presence and the size-hetero-
geneity of β-endorphin immunoreactivity in the ectopic ACTH/β-LPH-producing tumors using Sephadex G-50 gel chromatography, sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and a sensitive radioimmunoassay for β-endorphin recently established in our laboratory (Yoshimi et al., 1978).

Materials and Methods

Extraction and Radioimmunoassay Procedure
Six ectopic ACTH/β-LPH-producing tumors (3 medullary carcinomas of the thyroid, 2 malignant thymomas, and 1 oat cell carcinoma of the lung), which were obtained at autopsy or surgery and stored at -20°C until further processed, were extracted with a modification (Imura et al., 1973) of the method of Payne et al. (1950). To exclude the possibility of conversion from β-LPH to β-endorphin during the extraction procedures with acetic acid (Imura et al., 1973), 5 μg of βh-LPH (provided by Dr. W. E. Nicholson) was injected into 4 g of an ectopic ACTH/β-LPH-producing pancreatic tumor, which was then extracted along with the same amount of the tumor tissue without the addition of β-LPH in a similar way as described. The extract powder was reconstituted in 0.1 M acetic acid, centrifuged at 3,000 rpm for 30 min at 4°C and an aliquot of the supernatant using two fold dilutions was assayed for immunoreactive ACTH (Hirata et al., 1975b) and β-endorphin (Yoshimi et al., 1978) by each specific radioimmunoassay. In the present experiment, however, a new anti-β-endorphin serum (R1674) raised in a rabbit immunized with crude porcine ACTH preparations (Organon) was used. This antiserum was found to be directed to the 17-31 amino acid sequence of β-endorphin molecule and to react with human β-endorphin (βh-endorphin) and human β-LPH (βh-LPH) (both kindly provided by Dr. C. H. Li) on an equimolar basis.

Gel Chromatography
An aliquot of the supernatant of each tumor extract was applied on Sephadex G-50 superfine column (0.9 x 50 cm). The column was equilibrated and eluted with 0.1 M acetic acid at a flow rate of 5 ml/hr. Each 1 or 0.7 ml was collected and the content of β-endorphin immunoreactivity in the eluate was determined by a radioimmunoassay (Yoshimi et al., 1978). The recovery of 125I-βh-LPH and 125I-βh-endorphin applied on the column in different runs ranged from 70 to 80%.

SDS-polyacrylamide Gel Electrophoresis
In order to examine the molecular size of the big fraction with β-endorphin immunoreactivity, the eluates in the void volume after gel chromatography of the extract of a medullary carcinoma of the thyroid (case 6) were pooled, lyophilized and analyzed by SDS-polyacrylamide gel electrophoresis as previously reported (Yoshimi et al., 1978). After electrophoresis gels were cut into slices of 2 mm length and incubated with 0.5 ml of 5 mM NaHCO3 containing 0.05% SDS at 37°C for 8 hr; each eluate was assayed for β-endorphin immunoreactivity. Bovine serum albumin (BSA) (molecular weight 68,000), lactic dehydrogenase (LDH) (36,000), chymotrypsinogen (25,700), βh-LPH (provided by Dr. C. H. Li) and βh-endorphin were run as the markers and dyed with Coomassie Blue.

Results
Serial dilutions of the extracts of 4 ectopic ACTH/β-LPH-producing tumors were found to be parallel to the standard curve for βh-endorphin (Fig. 1), indicating that βh-endorphin activity present in the tumor extracts is not distinguishable from βh-endorphin immunologically. After Sephadex G-50 superfine gel chromatography, β-endorphin immunoreactivity of the tumor extracts appeared as two major peaks in all samples (Fig. 2); one eluted at a position where 125I-βh-LPH emerged and the other consistent with that of 125I-βh-endorphin. However, in one case of medullary carcinoma of the thyroid (case 6), another fraction eluting in the void volume (Vo) was apparent (Fig. 2).

After Sephadex gel chromatography of the extract equivalent to 0.2 g of an ectopic ACTH/β-LPH-producing pancreatic tumor, to which was exogenously added 5 μg of βh-LPH immediately before the extraction procedures, β-endorphin immunoreactivity appeared as one conspicuous peak that eluted in a position of 125I-βh-LPH (Fig. 3), indicating that there could be no significant conversion from βh-LPH to β-endorphin during the extraction procedures. The contents of total immunoreactive ACTH
and β-endorphin in the tumor were 2.0 and 2.1 ng/g wet weight, respectively, and there was no detectable β-endorphin immunoreactivity (less than 70 pg/fraction) after gel chromatography of the extract equivalent to 0.2 g of the tumor alone (Fig. 3).

In order to examine the molecular size of this largest component with β-endorphin immunoreactivity, the eluates in the void volume after the gel filtration of the extract of case 6 were pooled, lyophilized and analyzed by SDS-polyacrylamide gel electrophoresis as previously reported (Yoshimi et
Fig. 3. Fractionation of the extracts of an ectopic ACTH/β-LPH-producing pancreatic tumor with and without the addition of 5 μg of βh-LPH before the extraction procedures on a Sephadex G-50 superfine column (0.9 × 50 cm) eluted with 0.1 M acetic acid at 4°C with a flow rate of 5 ml/hr. Each 0.7 ml was collected and immunoreactive β-endorphin in each eluate was determined by a radioimmunoassay. The void volume (Vo) and the elution positions of 125I-βh-LPH and 125I-βh-endorphin were shown on top.

Fig. 4. Analysis of “big-big”-β-endorphin by SDS-polyacrylamide gel electrophoresis. The eluates containing the large molecules with β-endorphin immunoreactivity obtained from the extract of a medullary carcinoma of the thyroid (case 6) after gel filtration (Fig. 1) were pooled, lyophilized and analyzed by SDS-polyacrylamide gel electrophoresis. BSA (molecular weight 68,000), LDH (36,000), chymotrypsinogen (25,700), β-LPH and βh-endorphin were run as the markers and dyed with Coomasie Blue. The elution positions of these markers are shown on top.

al., 1978). After electrophoresis, 3 main peaks of β-endorphin immunoreactivity were resolved (Fig. 4). The biggest from of β-endorphin immunoreactivity appears to have an apparent molecular weight of approximately 36,000 according to the calibration with the markers in this gel system. The second and the third peaks seem to be best attributed to β-LPH and β-endorphin, respectively, on the basis of the relative
Table 1. Summary of ectopic ACTH/ß-LPH-producing tumors studied.

<table>
<thead>
<tr>
<th>Case</th>
<th>Tumor type</th>
<th>Immunoactive ACTH (ng/g tissue)</th>
<th>Immunoactive ß-endorphin (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Medullary carcinoma of the thyroid</td>
<td>78</td>
<td>23** (35)**</td>
</tr>
<tr>
<td>2</td>
<td>Malignant thymoma</td>
<td>184</td>
<td>340 (792)</td>
</tr>
<tr>
<td>3</td>
<td>Malignant thymoma</td>
<td>48</td>
<td>285 (486)</td>
</tr>
<tr>
<td>4</td>
<td>Medullary carcinoma of the thyroid</td>
<td>108</td>
<td>49 (83)</td>
</tr>
<tr>
<td>5</td>
<td>Oat cell carcinoma of the lung</td>
<td>94</td>
<td>71 (96)</td>
</tr>
<tr>
<td>6</td>
<td>Medullary carcinoma of the thyroid</td>
<td>77</td>
<td>42 (120)</td>
</tr>
</tbody>
</table>

Contents of immunoreactive ß-endorphin and ACTH in 6 ectopic ACTH/ß-LPH-producing tumors were expressed as ng per g wet weight of tissues. Because the anti-ß-endorphin serum (R 1974) used in the present experiment reacted with ßh-LPH on an equimolar basis, contents of immunoreactive ß-endorphin in the tumor tissues were determined by multiplying the directly-assayable immunoreactive ß-endorphin by the ratio of ß-endorphin to total ß-endorphin immunoactivity obtained from each elution profile of the extracts of tumors after Sephadex gel filtration (Fig. 2).

Discussion

We have demonstrated valid evidence for the concomitant production of ß-endorphin in 6 ectopic ACTH/ß-LPH-producing tumors using Sephadex gel chromatography and a specific radioimmunoassay for ß-endorphin. Our finding is quite in accord with those of Orth et al. (1977) and Bertagna et al. (1978) who observed the concomitant production of ACTH, ß- and ÿ-LPH, and ß- and ß-endorphin in a pancreatic islet cell carcinoma and in a pulmonary small cell carcinoma, respectively. The acetic acid extraction (Imura et al., 1973) used in the present study could be expected to generate ß-endorphin from the enzymatical degradation of ß-LPH (Liotta et al., 1978). However, the possibility that ßh-LPH contained in the tumors might be converted enzymatically to ßh-endorphin during the extraction is less likely because the conversion from ßh-LPH, exogenously added to the tumor, to ßh-endorphin was not demonstrated, as shown in Fig. 3, after gel chromatography of the extract. ß-LPH degrading enzymes contained in the tumor tissues might be inactivated by heating the homogenates at 70°C for 30 min immediately after homogenization of the tissues (Imura et al., 1973), which could be considered to inactivate completely proteolytic enzymes thereafter. Bachelot et al. (1977) also observed no enzymatical conversion to ß-MSH in the human pituitaries which were extracted with acetic acid but immediately heated in a boiling water bath for 3 min after the extraction procedure. ß-Endorphin was also reported to be synthesized in the mouse pituitary tumor cell lines (Mains et al., 1977; Allen et al., 1978) and the bovine (Crine et al., 1977) and rat (Crine et al., 1978) pituitary glands in vitro. However, the possibility of slight conversion from ß-LPH to ß-endorphin during extraction procedures could not be entirely excluded.

Amounts of ß-endorphin in the tumors were nearly comparable to those of ACTH although the relative values of two hormones seemed to vary among the tumors.

Mobilities in the gel although other smaller peaks were also present.

Contents of immunoreactive ACTH and ß-endorphin in 6 ectopic ACTH/ß-LPH-producing tumors studied are summarized in Table 1. ß-Endorphin contents of the tumor tissues were calculated by multiplying the values of directly-assayable immunoreactive ß-endorphin by the ratio of ß-endorphin to total ß-endorphin immunoactivity obtained from each elution profile of the extracts of tumors on Sephadex G-50 gel chromatography (Fig. 2). Amounts of ß-endorphin in the tumors were approximately comparable to those of ACTH although the relative values of both hormones seemed to vary among the tumors.
(Table 1). Mains et al. (1977) also reported simultaneous synthesis and release of approximately equimolar amount of ACTH and \( \beta \)-endorphin-like peptides from the At T-20/D-16v mouse pituitary cells.

In one case of medullary carcinoma of the thyroid (case 6), a large component of \( \beta \)-endorphin immunoreactivity with an apparent molecular weight of approximately 36,000 was demonstrated, in addition to \( \beta \)-LPH, \( \beta \)-endorphin and a material with \( \beta \)-endorphin immunoreactivity which moved faster than \( \beta \)-endorphin, by Sephadex gel chromatography and subsequent SDS-polyacrylamide gel electrophoresis. It is puzzling that the big fraction of \( \beta \)-endorphin immunoreactivity obtained from gel chromatography was shown to contain \( \beta \)-LPH, \( \beta \)-endorphin-like peptides and a smaller material with \( \beta \)-endorphin immunoreactivity besides a larger molecular component after gel electrophoresis. This finding, however, could be explained by the size-heterogeneity of peptides fractionated by gel chromatography probably due to aggregation and/or non-specific binding to larger proteins. Degradation of the big molecular components to the smaller peptides during gel electrophoresis seems to be unlikely to occur because the samples to be electrophoresed were heated at 100°C for 3 min in a boiling water bath in order to be enzymatically deactivated.

We reported previously the presence of "big-big" \( \beta \)-endorphin ("big"-\( \beta \)-LPH) with an apparent molecular weight of approximately 36,000 in the human pituitary extract (Yoshimi et al., 1978). Both the largest component of \( \beta \)-endorphin immunoreactivity in ectopic ACTH/\( \beta \)-LPH-producing tumors (the present study) and human pituitary gland (Yoshimi et al., 1978) seems to be identical to the common precursor to ACTH and \( \beta \)-endorphin-related peptides proposed by Mains et al. (1977) and Roberts and Herbert (1977) since ACTH antigenicity has been also detected in this component (Yoshimi et al., 1978; Sueoka et al., unpublished data). The presence of a common precursor has also been observed in the pituitary gland of the cow (Nakanishi et al., 1977) and the rat (Crine et al., 1978) as well as in the ectopic ACTH/\( \beta \)-LPH-producing tumors (Orth et al., 1977; Bertagna et al., 1978). Moreover, a common precursor was also reported to be present in the human placenta (Nakai et al., 1978; Odagiri et al., 1979).

From these findings it is suggested that ectopic ACTH/\( \beta \)-LPH-producing tumors and pituitary glands of several species including man synthesize ACTH as well as \( \beta \)-LPH/\( \beta \)-endorphin-related peptides in a quite similar way, all deriving from the common precursor.

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References


