Immunoreactive β-Endorphin in the Rat Mammatropic Transplantable Tumor (MtT-F4)

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ABSTRACT

Raised levels of what appeared to be β-lipotropin (β-LPH), β-melanotropin hormone, and β-endorphin were detected by radioimmunoassay in the plasma of rats bearing the mammatropic transplantable pituitary tumor MtT-F4. The immunoreactivity to anti-β-endorphin in the assay was displayed by a substance with the molecular weight of β-LPH, as determined by gel filtration. Isolated cells of MtT-F4 tumor incubated in vitro released immunoreactive β-LPH and β-endorphin, with the expected molecular weights, into the incubation medium. These results suggest that the pituitary transplantable rat tumor MtT-F4 secretes peptides structurally related to β-LPH.

INTRODUCTION

The transplantable pituitary tumor MtT-F4 was described by Furth (10) in 1954. This tumor secretes large quantities of prolactin, growth hormone, and ACTH (1, 22). The transplantable mouse pituitary tumor AIT-20, known for its high ACTH secretion, produces peptides similar to β-MSH (21, 25). The presence of peptides similar to β-LPH and β-MSH has been suggested in a human nonpituitary tumor secreting large quantities of ACTH (14).

β-LPH is a 91-amino acid peptide that contains the complete β-MSH sequence. It has therefore been postulated (4) that β-LPH is a precursor of β-MSH. The structure of endorphins (13, 16) suggests that β-LPH could be a precursor for these morphinomimetic peptides, which have been demonstrated in many species (6, 11).

The hypothesis has been advanced (Refs. 19 and 20; unpublished results) that ACTH and β-LPH are synthesized in the pituitary as constituents of a common precursor molecule. This hypothesis would be strengthened if it could be established that an ACTH-secreting tumor produces β-LPH and structurally related peptides such as β-MSH and β-endorphin. We examined the MtT-F4 tumor with this in mind.

MATERIALS AND METHODS

Tumor Transplantation. The MtT-F4 tumor was obtained from Dr. A. E. Bogden, Mason Research Institute, Worces-
ter, Mass., and had been passaged at least 10 times before the experiments described. The transplantation procedure is described elsewhere (18). We used 30 male Fischer 344 rats, 15 were transplanted with the tumor and 15 were sham-operated to serve as controls. All the animals received Purina laboratory chow and tap water ad libitum. Illumination periods were 12 hr long.

Plasma. Thirty days after transplantation, blood was collected by puncture of the abdominal aorta under ether anesthesia. The blood was collected in tubes that contained EDTA (0.07 ml of a 15% solution) and centrifuged at 4000 rpm and 4° for 20 min. The plasma was immediately separated, frozen in 250-μl portions, and stored at −60° until radioimmunoassay.

Incubation of Isolated Tumor Cells. A suspension of tumor cells was obtained by collagenase digestion (18). The isolated cells were incubated for 5 hr at 37° in 25 ml of Earle's balanced salt solution (9) containing 1.25 mg of lima bean trypsin inhibitor (Sigma Chemical Co., St. Louis, Mo.), 1.25 mg of bacitracin (Sigma), and 25 mg of bovine serum albumin (Sigma). After the incubation period, the suspension was centrifuged at 2000 rpm for 10 min, and the supernatant was collected, lyophilized, and stored for radioimmunoassay.

Gel Filtration. Plasma samples (250 μl) and reconstituted incubation medium (1 ml) were each applied to a Sephadex G-50 superfine column (1 cm x 35 cm) equilibrated with 0.01 M phosphate buffer (pH 7.6) containing 0.15 M NaCl, 0.025 M EDTA, 0.001% Merthiolate, and 1% bovine serum albumin. Human 125I-labeled β-MSH, sheep 125I-labeled β-LPH, and sheep 125I-labeled β-endorphin were used to calibrate the column. Radioimmunoassay was performed on each fraction.

Radioimmunoassay. Antiserum to porcine β-MSH was raised in a guinea pig and kindly supplied by Dr. M. Donnadieu (8). Laboratoire d'Exploration Fonctionnelle, Hôpital Trousseau, Paris, France. Antiserum to ovine β-LPH was raised in New Zealand White rabbits by the standard immunization procedure with 200 μg of ovine β-LPH per rabbit and Freund's complete adjuvant. Before the immunization, the peptide was conjugated to ovalbumin (carboxi-
diimide method) by Dr. Peter W. Schiller of this Institute. Antiserum to synthetic ovine β-endorphin was kindly provided by Dr. Roger Guillemin, The Salk Institute, La Jolla, Calif. The immunization procedure has been published (2).

The peptides used as standards or tracers (ovine and human β-LPH, ovine β-LPH, ovine β-endorphin, and human and porcine β-MSH) were extracted and purified by carboxymethyl cellulose chromatography as described elsewhere (23). Rat growth hormone and rat prolactin were generously supplied by the National Institute of Arthritis and Metabolic Diseases rat pituitary hormone program. Synthetic ACTH1-24 was kindly provided by Dr. W. Rittel, Ciba-Geigy, Basle, Switzerland, and synthetic α-MSH was supplied by Dr. Peter W. Schiller.
The method of Hunter and Greenwood (15), slightly modified, was used to iodinate the peptides with 125I. The antibody-antigen complex was precipitated with polyethylene glycol (7), and the precipitated radioactivity was measured in a Beckman g-300 counter (Beckman Instruments, Inc., Fullerton, Calif.). All measurements were done in duplicate.

Specificity of the Antisera. Antiserum to porcine β-MSH was tested against various peptides with human β-MSH purified in our laboratory as a tracer. Purified human β-LPH gave about 17% cross-reactivity, with a curve nearly parallel to the standard (Chart 1). There was no cross-reactivity with human β-endorphin, synthetic ACTH₁₋₄₉, α-MSH (Ciba-Geigy), rat growth hormone, or rat prolactin.

Antiserum to ovine synthetic β-endorphin gave no cross-reactivity with synthetic ACTH₁₋₄₉, rat prolactin, rat growth hormone, α-MSH (Ciba-Geigy), or human β-MSH when ovine β-endorphin was used both as a tracer and as a standard. There was, however, parallel displacement with β-LPH (Chart 2). Antiserum to ovine β-LPH with ovine β-LPH₄₋₁₆ as tracer and standard showed parallelism between ovine β-LPH and γ-LPH. β-MSH did not react (Chart 3).

Statistics. The concentration of circulating hormones in plasma (Table 1) is given as the arithmetic mean ± S.E. The difference between the tumor-bearing animals and the controls was evaluated by the 2-sample rank testing of Mann and Whitney (Ref. 26, pp. 109-110).

The standard curve was linearized by the logit transformation. All the calculations and transformations were performed on a Hewlett-Packard Model 9810 calculator (Hewlett-Packard, Cupertino, Calif.).

The correlation coefficients for the tumor weight versus level of circulating hormones were calculated as described by Zar (Ref. 26, p. 237), and the significance of r was evaluated by the t test.

RESULTS

Table 1 gives the plasma concentrations of β-LPH, β-MSH, and β-endorphin in control rats and in those with MIT-F4 tumors 30 days after tumor transplantation. For all 3 hormones assayed, the values were significantly higher in the tumor-bearing animals than in the controls. Of the 3 hormones, β-endorphin, at least as estimated by radioimmunoassay, seemed to be present in the highest concentration.

The correlation between plasma β-endorphin concentration and tumor weight 30 days after transplantation is shown in Chart 4. The correlation coefficient was 0.75 (p < 0.001). The correlation between β-MSH plasma concentrations and the tumor weight was not remarkable (coefficients of 0.59 and 0.25, respectively).

Chart 5 shows the results of gel filtration experiments. In plasma all the β-endorphin immunoreactivity was eluted at a molecular weight corresponding to β-LPH, whereas immunoreactive β-endorphin from the incubation medium was eluted from the Sephadex column in 2 peaks, corresponding to β-LPH and β-endorphin elution volumes. β-MSH data likewise indicated the absence of β-MSH from the plasma of tumor-bearing rats; all the β-MSH immunoreactivity was eluted mostly at volumes corresponding to β-LPH with a small peak corresponding to γ-LPH elution volume (unpublished results). After gel filtration of the incubation medium, we could not detect β-MSH immunoreactivity. The β-LPH antiserum detected immunoreactive β-LPH both in the incubation medium and in the plasma.

Competition curves with various dilutions of plasma and incubation medium (Chart 6) are parallel with the ovine β-endorphin as standard and tracer in the β-endorphin radioimmunoassay.

DISCUSSION

The pituitary transplantable tumor MIT-F4 was shown to secrete peptides structurally related to β-LPH, in agreement with the results for the mouse tumor AtT-20 (20), which secretes peptides structurally related to β-MSH (21), and for
M. Bourassa et al.

Chart 3. Anti-β-LPH radioimmunoassay displacement curves, with ovine β-LPH as tracer and standard, for ovine β- and γ-LPH. Ovine β-MSH did not react. For ordinate formula see Chart 1.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>β-Endorphin (ng/ml)</th>
<th>β-MSH (ng/ml)</th>
<th>β-LPH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 14)</td>
<td>4.89 ± 0.68</td>
<td>0.34 ± 0.31</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td>MtT-F4 (n = 13)</td>
<td>15.6 ± 3.62</td>
<td>0.86 ± 0.12</td>
<td>0.60 ± 0.074</td>
</tr>
</tbody>
</table>

*a* Means ± S.E.  
*b* p < 0.01.

Chart 4. Correlation between tumor weight and the apparent plasma β-endorphin concentration in rats bearing the MtT-F4 tumor for 30 days. The endorphins were measured by radioimmunoassay with the use of anti-β-endorphin serum, with ovine β-endorphin as tracer and standard.

some human ACTH-secreting tumors that are reported to secrete peptides related to β-LPH (14). The secretion of such peptides seems to be common to at least 3 different ACTH-producing tumors in 3 different species.

The β-LPH molecule in pigs (12, 13), sheep (3), and humans (17) had a constant COOH-terminal fragment (including the β-endorphin 61 to 91 sequence) with a variation in only 1 position; while the NH2-terminal fragment, identified as γ-LPH (amino acids 1 to 58), shows many differences in amino acid composition from 1 species to the other. These differences are obviously important for the interpretation of our radioimmunological study and could explain why the radioimmunoassay indicated a higher concentration of β-endorphin than that of β-MSH and β-LPH. Because of the apparent constancy of the COOH-terminal fragment, we can expect rat β-endorphin to be more reactive with the antisera raised with ovine β-endorphin than rat β-MSH would be to anti-porcine β-MSH or rat β-LPH to anti-ovine β-LPH. Despite these problems of possible species specific-

Chart 5. Competition curves with dilutions of plasma and incubation medium in the β-endorphin radioimmunoassay. Ovine β-endorphin was used as tracer and standard. The curves for plasma and incubation medium have been arbitrarily placed along the abscissa to clarify the results. For ordinate formula see Chart 1.
Acknowledgments

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References

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