BETA-ENDORPHIN AND BETA-LIPOTROPIN SECRETION BY AN ACTH-SECRETING MOUSE PITUITARY TUMOR

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1. Introduction

The discovery of the morphine-like peptide, beta-endorphin, which is identical to the sequence 61–91 of beta-lipotropin (beta-LPH) [1–4] gave additional support to the idea that beta-LPH is a prohormone as suggested [5–7]. In vitro biosynthetic experiments have demonstrated the production by pituitary tissue of beta-endorphin, beta-LPH, and gamma-LPH (beta-LPH 1-58) [8–10]. It has been also suggested that an ACTH-secreting mouse pituitary tumor cell line (AtT-20/D-16V) synthesizes beta-LPH as a part of a larger precursor protein which contains the sequence of ACTH [11–13]. We demonstrate here the existence of immunoreactive beta-endorphin in the plasma of AtT-20 tumor-bearing mice and in extracts of these tumors.

2. Materials and methods

2.1. *Preparation of tumor extract and plasma*

The AtT-20 tumor [14,15], obtained from Dr A. E. Bogden (Mason Research Institute, Worcester, MA), was periodically retransplanted in adrenalectomized LAF_1 mice. Two tumors (total wet wt 280 mg) were removed from mice killed by decapitation and immediately homogenized in 2 ml ice-cold 1 mM EDTA (pH 10.3), containing bovine serum albumin (fraction V, GIBCO) (1 mg/ml) and bacitracin (Sigma Chemical) (500 μg/ml) by a Teflon®–glass homogenizer. After centrifugation (15 min, at 15 000 rev./min, 4°C), the supernatant was frozen at –20°C.

Trunk blood was collected into ice-cold tubes containing EDTA and aprotinin (Trasylol®, Boehringer Ingelheim Canada Ltd) (final concentrations: 2 mg and 500 kIU/ml blood, respectively). Plasma was separated by centrifugation (10 min, 4000 rev./min, 4°C), and frozen at –20°C. Plasma from adrenalectomized LAF_1 mice was used as control. Both tumor extract and plasmas were stored at –20°C, until further studies.

2.2. *Beta-endorphin radioimmunoassay*

The details of our procedure have been described [16,17]. Antiserum against beta-endorphin was kindly supplied by Dr Roger Guillemin, Salk Institute, LaJolla, CA. It cross-reacts with beta-LPH (58% on a molar basis) (fig.1A), but does not with gamma-LPH, beta-MSH, ACTH or alpha-MSH. Ovine beta-endorphin, isolated and purified as in [18], was used as tracer and standard. Tumor extract and plasma samples were assayed in duplicate at several dilutions. The displacement curves were linearized by log-logit transformation. Weighted least squares regression lines were fitted and parallel-line potency estimates made by the procedure in [19].

2.3. *Gel filtration*

Samples of tumor extract (10 μl) or plasma of tumor-bearing mice (200 μl) were applied to a 1 X 55 cm

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Fig.1a

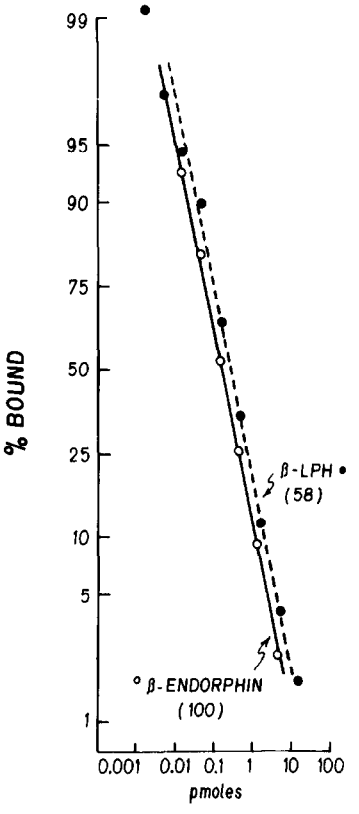

Fig.1b

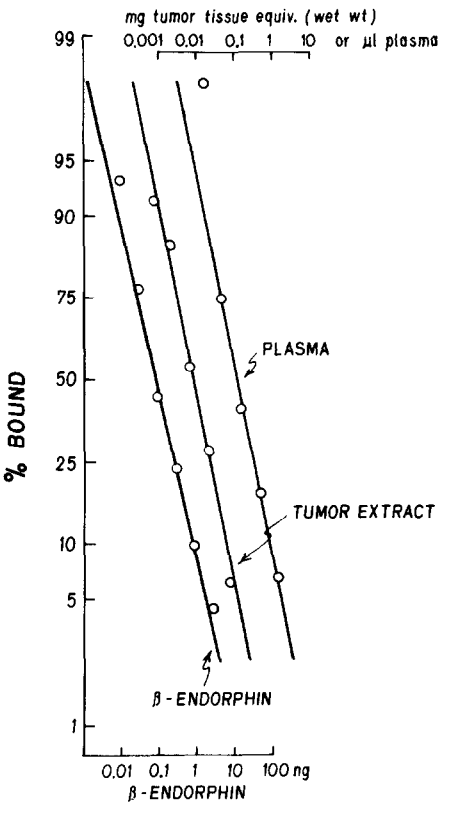

Fig.1a. Anti-beta-endorphin serum specificity: cross-reactivity with beta-LPH. Fig.1b. Displacement curves for tumor extract and plasma of tumor-bearing adrenalectomized mice parallel to the standard curve.

Sephadex® G-50 superfine column, equilibrated and eluted with 0.01 M phosphate, 0.15 M NaCl, 0.025 M EDTA, 0.001% merthiolate, 1% bovine serum-albumin, pH 7.6, buffer. One ml fractions were collected and assayed for beta-endorphin immunoreactivity. The column was calibrated with ferritin (void volume), ^{125}I-labeled beta-LPH and ^{125}I-labeled beta-endorphin.

3. Results

As illustrated in fig.1B, immunoreactive (IR)-beta-endorphin is present in AtT-20 tumor extracts as well as in plasma of tumor-bearing mice. Both displacement curves were parallel with the standard beta-endorphin. The tumor tissue contains 11.3 ng/mg wet wt (95% confidence interval, 10.9–11.7) and the plasma 785 ng/ml (760–810) of IR beta-endorphin. Plasma of adrenalectomized mice contains 4.5 ng/ml (4.3–4.7).

Figure 2 shows the molecular weight distribution

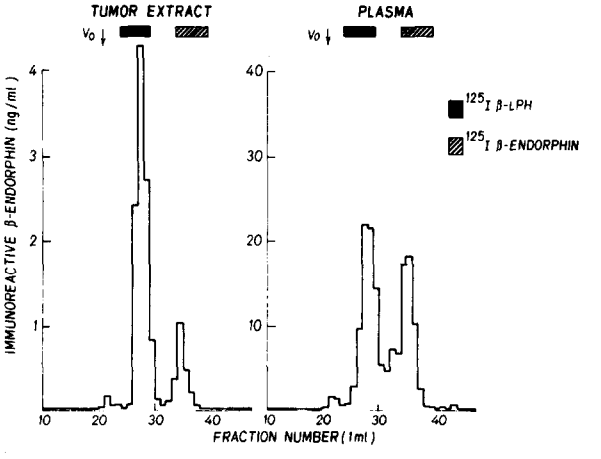

Fig.2. Elution profiles of immunoreactive beta-endorphin from Sephadex G-50 superfine chromatography for AtT-20 tumor extract and plasma of AtT-20 tumor-bearing adrenalectomized mice. Elution volumes of beta-LPH and beta-endorphin are shown.

of IR beta-endorphin in both tumor extract and plasma, as obtained by gel filtration on a Sephadex G-50 superfine column. Two major peaks are separated; the first one corresponds to the elution volume of ovine beta-LPH and the second one to beta-endorphin. The relative size of these 2 peaks differs in tumor extract and plasma samples.

4. Discussion

This study provides evidence that the AtT-20 tumor secretes immunoreactive beta-endorphin. The AtT-20 tumor has been shown to contain different forms of ACTH [20–22] and MSH [20]. Likewise, the production of immunoreactive beta-endorphin by the rat transplantable pituitary tumor MtT-F4 which is known to secrete ACTH has been described [17]. Immunocytochemical studies have pointed out the presence of ACTH, beta-LPH and beta-endorphin in the same cells of anterior and intermediate pituitary lobes [23,24]. The biosynthesis of a common precursor (31 K) to beta-endorphin and ACTH [10–12], as well as beta-endorphin and ACTH-like peptides was demonstrated by experiments using the AtT-20/D-16V cell line [25]. Our results support the concept of a concomitant secretion of beta-endorphin and ACTH [26].

The immunoreactive beta-endorphin secreted by AtT-20 tumors appears to be present in 2 major molecular forms. The first one has a molecular weight corresponding to that of standard ovine beta-LPH, while the second one corresponds to beta-endorphin itself. In agreement with structural relationship and common immunoreactivity of beta-LPH and beta-endorphin, it is reasonable to believe that the higher molecular weight component is in fact beta-LPH. The precursor nature of this higher molecular weight component is also suggested by the relative increase of the lower molecular weight component in plasma as compared to tumor tissue extract. This relative increase of beta-endorphin in plasma may be a consequence of preferential secretion of the smaller peptide and/or peripheral cleavage of beta-LPH to beta-endorphin. On one hand, the latter alternative is supported by in vivo demonstration of cleavage of beta-LPH into beta-endorphin in rabbits injected with purified ovine beta-LPH [27]. On the other hand, AtT-20/D-16V cultured cells [11,25] and AtT-20 tumor isolated cells (unpublished results) synthesize beta-endorphin and release it into incubation medium in relatively low concentrations. Secondary degradation of beta-LPH during manipulation of the plasma is very improbable because all studies were performed at 4°C with a protease inhibitor (Aprotinin). It was also demonstrated in rabbits [27] that beta-LPH has a significantly lower disappearance rate than beta-endorphin. This could explain persistent beta-LPH-like material in plasma.

The presence of a relatively low peak of high molecular weight IR-beta-endorphin (fig.2) in plasma and in tumor extract could be explained either by some degree of aggregation or by the presence of a 20–30 K precursor form of both ACTH and beta-LPH [21,22].

The high level of beta-endorphin in the plasma of AtT-20 tumor-bearing mice might influence pain sensitivity and induce a state similar to opiate tolerance-dependence [28,29]. This would require passage of the peptide from the circulation of the brain across the blood–brain barrier to reach central nervous system opiate receptors. Such a phenomenon has been suggested by the observation of analgesia following intravenous injection of a high dose of beta-endorphin in mice [30]. In rabbits, beta-endorphin crosses the blood-brain barrier into the CSF [27]. An increase in brain beta-endorphin levels could be expected from this hypothesis, although the absence of a parallel between blood and brain levels of beta-endorphin immunoreactivity has been established following adrenalectomy in rat [31]. The behavior of these tumor-bearing mice is not grossly affected.

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