Chromatographic characteristics of pro-opiomelanocortin-derived peptides from the rat transplantable tumour 7315a

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ABSTRACT

Adrenocorticotropic (ACTH) and other pro-opiomelanocortin (POMC)-derived peptides produced by the 7315a corticomammatrophic tumour have been poorly studied although they elicit profound hypertrophy and hyperplasia in the adrenal glands of recipient Buffalo rats. Tumour extracts were chromatographed on Sephadex G-75 and fractions monitored for POMC-derived peptides by four radioimmunoassay (RIA) systems: ACTH, α-MSH, β-lipotrophin (β-LPH)/endorphin and N-terminal POMC (N-POMC). Chromatograms were compared with those of pars distalis extracts from normal Buffalo rats. All four RIA systems detected immunoreactive material in tumour extracts. ACTH, β-LPH/endorphin and N-POMC were present in approximately equimolar amounts (ACTH content 93±40±5.27 (S.E.M.) pmol/g) whereas α-MSH was present in smaller amounts (2.83±0.13 pmol/g). Total peptide content correlated well with tumour weight. ACTH immunoreactivity (IR) in Sephadex chromatograms was located in a large 20 000 mol. wt peak, an ACTH(1–39) peak and a smaller peak coinciding with ACTH(1–24). The latter two peaks showed biological activity consistent with ACTH(1–39) and an ACTH (1–24)-like peptide respectively. The β-LPH/endorphin RIA revealed a peak eluting at approximately 20 000 mol. wt which could not be ascribed to any known POMC peptide containing the endorphin sequence. A β-LPH-like peak, a β-endorphin-like peak and a smaller-sized peak, which contained the bulk of the β-LPH/endorphin IR, were detected; the low molecular weight peak probably representing α- or γ-endorphin. The N-POMC RIA revealed a 20 000 mol. wt peak and a wide peak which could not be completely resolved into two peaks, and which probably represented N-POMC(1–95) and N-POMC(1–74). No 30 000 mol. wt precursor could be detected with any of the RIA systems employed. Sephadex chromatography of material released from perfused dispersed tumour cells revealed identical IR peaks with all RIA systems used. Glycosylation of tumour POMC peptides was assessed by Concanavalin A–agarose (Con A) chromatography of pooled IR peaks from Sephadex chromatograms. The 20 000 mol. wt (both IR-ACTH and IR-N-POMC) peak, IR-N-POMC(1–95) and IR-N-POMC(1–74) peaks all bound to Con A and were specifically eluted with methylglucoside. Sephadex G-75 and Con A chromatography of pooled pars distalis extracts from normal Buffalo rats were performed. They showed significant differences, compared with tumour extract chromatograms, with all the RIA systems employed. It is concluded that the 7315a tumour processes POMC in a different manner when compared with normal Buffalo rat pars distalis and that it may be used as an interesting model in which to study the processing of POMC. In addition it may shed light on the role of POMC-derived peptides, other than ACTH(1–39), on adrenal growth and function.


INTRODUCTION

The production of adrenocorticotropic (ACTH) and related peptides by pituitary and non-pituitary tumours in man has been well documented (Hirata, Yamamoto, Matsukura & Imura, 1975; Oki, Nakao, Tanaka et al. 1981). In rats and mice there are few well-established tumour cell lines and transplantable tumours which produce and secrete ACTH (Cohen & Kim, 1963; Nakane, Sétáló & Mazurkiewicz, 1977).
Pro-opiomelanocortin (POMC) processing was originally studied using the mouse pituitary tumour cell line AtT20/D-16v as a model (Roberts, Phillips, Rosa & Herbert, 1978; Mains & Epiper, 1981). In the rat there are some tumour lines (e.g. MtTF4 and 7315a) which produce and secrete ACTH, alone or in combination with other hormones, such as prolactin and/or growth hormone (Bates, Garrison & Morris, 1966). The production of prolactin by the rat transplantable tumour 7315a has been extensively studied in vivo and in vitro (Lamberts, Nagy, Uitterlinden & MacLeod, 1982; Judd, Koike, Schettini et al. 1985). The original report on the production of ACTH by the 7315a tumour was by Bates et al. (1966) and this was followed by information about ACTH and other POMC peptides produced by this tumour (Lamberts, MacLeod & Krieger, 1980), including some information on tumour lipotrophin (LPH) and endorphin content (Panerai, Sawynok, Labella & Friesen, 1980). It has been reported that the 7315a tumour induces profound changes in the adrenal cortex (i.e. hypertrophy and hyperplasia) which have been ascribed to ACTH (MacLeod & Lehmeyer, 1973). In view of the recently described effects of other POMC-derived peptides on the growth and function of the adrenal cortex (Pedersen, Brownie & Ling, 1980; Al-Dujaili, Hope, Estivariz et al. 1981; Matsuoka, Mulrow, Franco-Saenz & Li, 1981; Estivariz, Iturriza, McLean et al. 1982; Lowry, Silas, McLean et al. 1983), we have analysed chromatographically the production of several peptides derived from different regions of POMC in the 7315a tumour as a first step towards explaining the effects exerted by the tumour on the hypothalamo-pituitary-adrenal axis. In addition the work may provide valuable information about the mechanism involved in POMC processing.

**MATERIALS AND METHODS**

**Reagents**

All reagents used were of analytical grade. Corticosterone, collagenase type IV, deoxyribonuclease I type DN-100, trypsin type III, lima bean trypsin inhibitor, bovine serum albumin (BSA) (radioimmunoassay (RIA) grade), molecular weight markers for gel filtration chromatography, phenylmethyl-sulphonyl-fluoride (PMSF), Polyep, methyl glucopiranoside, Concanavalin A-agarose and dextran (mol. wt 70 000) were provided by Sigma, St Louis, MO, U.S.A. Sephadex G-75 Superfine and chromatography columns were from Pharmacia, Uppsala, Sweden; Biogel P-2 (200–400 mesh) was from Bio Rad, Richmond, CA, U.S.A. and Norit A-activated charcoal from Pablo Zubizarreta Ward, Buenos Aires, Argentina. Synthetic human ACTH(1–39), ACTH(1–24), ACTH(17–39) and α-melanocyte-stimulating hormone (α-MSH) were kindly supplied by Drs P. A. Desaulles and W. Rittel, Ciba-Geigy, Basle, Switzerland. Synthetic N-terminal POMC(1–48) (N-POMC(1–48)) was a gift from Dr J. Ramachandran, Genentec Inc., San Francisco, CA, U.S.A. Human N-POMC(1–76) (hN-POMC(1–76)) and hβ-LPH were purified at St Bartholomew's Hospital, London and kindly donated by Dr P. J. Lowry as was rabbit N-POMC antisera R2BH and PS4B2 and C-terminal ACTH antiserum. γ3-MSH was a gift from Dr P. Owens, University of Newcastle, New South Wales, Australia. Human β-endorphin and (Phe3, Nle4)ACTH(1–24) were from Peninsula Laboratories, Palo Alto, CA, U.S.A. Rabbit anti-α-MSH serum and synthetic desacetyl-α-MSH were a gift from Dr A. Eberle, Institute of Molecular Biology and Biophysics, Zurich, Switzerland, and rabbit anti-β-endorphin serum E-286 was donated by Dr E. Spinedi, CRRIE, La Plata, Argentina. Rabbit midportion and N-terminal ACTH antiserum and goat anti-rabbit sera were produced in our laboratory. Rat prolactin RIA materials were supplied by the NIADDK Rat Pituitary Hormone Distribution Program. Radiodione and 45CaCl2 were from New England Nuclear, Boston, MA, U.S.A. Radiiodinated ACTH (1–39) was from Radioassay Systems Laboratories Inc., Carson, CA, U.S.A. Vycor glass was from Corning Glass Works, New York, NY, U.S.A. Eagle Hela and TC 199 media and fetal calf serum were from Difco, Detroit, MI, U.S.A. Penicillin and streptomycin were supplied by Lepetit Laboratories, Buenos Aires, Argentina.

**Biological specimens**

The mammatrophic and corticotrophic tumour 7315a was kindly provided by Dr R. M. MacLeod from the Department of Medicine, University of Virginia School of Medicine, Charlottesville, VA, U.S.A. in 1981 and maintained in our laboratory since then by successive transfer in Buffalo rats originally obtained from Simonsen Labs, Gilroy, CA, U.S.A. and subsequently bred in our laboratory. Transplants were performed as described previously (MacLeod, Smith & DeWitt, 1966) by s.c. inoculation of minced tumour in the suprascapular region of inbred adult female Buffalo rats. Alternatively, collagenase-dispersed tumour cells (approximately 107 cells) (Castro, Estivariz & Iturriza, 1984) were suspended in culture medium and injected subcutaneously in the same region. Animals were maintained in normal laboratory conditions with water and food available ad libitum. The tumour became palpable 15 and 25 days after inoculation of pieces or cells respectively. Tumour pieces or collagenase-dispersed tumour cells
from each generation transfer were suspended in Eagle’s HeLa medium containing 2% (v/v) fetal calf serum and 10% (v/v) dimethylsulphoxide, gradually cooled to \(-80^\circ\text{C}\) over a period of 48 h and kept at that temperature. Pituitary and adrenal glands were routinely extracted, weighed and either homogenized or fixed in Bouin’s fluid for histology.

**Peptide extraction and chromatography**

Tumour tissue was removed from rat hosts between days 15 and 50 after inoculation. Animals were killed and the tumour rapidly excised from surrounding normal tissue, weighed, chopped with a pair of scissors and rapidly homogenized in either 20 or 200 mmol HCl/l, acetic acid (1 mol/l) with PMSF (1 mmol/l), acetic acid (0-2 mol/l) or 1% (v/v) HCOOH (1 g tumour/2.5 ml acid mixture) with or without brief boiling to inactivate proteolytic enzymes. Tumour homogenates were immediately centrifuged at 12 000 g at 0 °C for 60 min. Pars distalis extracts from normal adult male Buffalo rats were processed in conditions similar to those for the tumour tissue. Supernatants from freshly obtained tumour extracts, frozen tumour extracts or pars distalis extracts were applied (approx. 5–10 ml) to a Sephadex G-75 Superfine column (3 × 80 cm), carefully calibrated with the following peptides and proteins: BSA, rat prolactin, hß-LPH, cytochrome C, \(\beta\)-lactalbumin, purified hN-POMC(1–76), synthetic N-POMC(1–48), ACTH (1–39), ACTH(1–24), \(\beta\)-endorphin, \(\alpha\)-MSH and \(45\text{CaCl}_2\). Runs were performed at room temperature using 1% (v/v) formic acid as eluant with an upward flow rate of 6 ml/h, collecting fractions every 20 min. Fractions were frozen at \(-20^\circ\text{C}\) unless immediately processed for RIA or bioassay. When supernatants of tumour cell incubates were run through the column, it was previously equilibrated with 0-1% (w/v) BSA and 1% (w/v) Polypep to minimize peptide adsorption to the column. Chromatography was performed three times with the same tumour extract, variations in retention times being less than 3% from run to run. Tumour cell perfusions were performed with freshly dispersed tumour cells packed in a small column together with Biogel P-2 (Gillies, Ratter, Grossman et al. 1980) and perfused at a slow rate with TC 199 medium added with 0-25% BSA. Cell column eluates were collected in 1 mol HCl/l containing 1-6% (w/v) glycine and either frozen or immediately subjected to Sephadex G-75 chromatography. Fractions collected were lyophilized and dissolved in RIA incubation buffer for peptide RIA.

**Radioimmunoassays**

The ACTH RIA was performed as described previously (Estivariz & Iturriza, 1985). Radioiodinated (Phe², Nle⁴)ACTH(1–24) was used as tracer. An antibody which recognizes the middle portion of ACTH was used (Castro et al. 1984) as it cross-reacts less than 0-1% with \(\alpha\)-MSH or desacetyl-\(\alpha\)-MSH and has no cross-reaction with ACTH(17–39). An N-terminal ACTH antisera which fully cross-reacts with ACTH (1–39), ACTH(1–24) and \(\alpha\)-MSH but not with ACTH (17–39) was used (Estivariz & Iturriza, 1985). A C-terminal ACTH antisera which cross-reacts fully with ACTH(1–39) and ACTH(17–39) and cross-reacts less than 0-05% with ACTH(1–24) was also employed. For the C-terminal ACTH RIA, \(^{125}\text{I}\)-labelled ACTH(1–39) was used as tracer (Jackson & Lowry, 1980). The \(\alpha\)-MSH RIA was performed according to Estivariz & Iturriza (1985), using an antibody provided by Dr A. Eberle which reacts 100% with desacetyl-\(\alpha\)-MSH but does not cross-react with ACTH or ACTH(1–24). The hß-LPH/endorphin RIA (Estivariz & Iturriza, 1985) used a \(\beta\)-endorphin antisera which cross-reacts fully with pure hß-LPH and shows less than 0-01% cross-reactivity with ACTH, \(\alpha\)-MSH, \(\beta\)-MSH and \(\gamma_3\)-MSH. The N-POMC RIA was performed as described previously (Hope, Ratter, Estivariz et al. 1981). Two antibodies (R2BH and PS4B2) against hN-POMC(1–76) were used; they both cross-react about 40% with N-POMC(1–48) but not with \(\gamma_3\)-MSH. The rat prolactin RIA was performed using the NIADDK RIA kit. Concanavalin A–agarose chromatography was performed as described by Iturriza & Estivariz (1986) using 0.5 × 3 cm agarose columns and methyl-glucopyranoside (0.5 mol/l) for elution of glycoproteins.

**ACTH bioassay**

Adrenocorticotrophin bioassay of tumour extracts or column fractions was accomplished using dispersed rat adrenal cells according to Lowry, McMartin & Peters (1973).

**RESULTS**

All results were obtained with tumour from 10–48 generation transfers in our laboratory. Tumour growth was uniform and reproducible from rat to rat, provided that the mass of tumour (or number of cells) inoculated was kept constant and non-necrotic or haemorrhagic areas of tumour were discarded before injection (Herreros, Morano & Iturriza, 1985). Tumour growth was linear from day 15 to day 50 after inoculation. Adrenal weight at autopsy showed marked hypertrophy, proportional to tumour size (total adrenal weight 238.29 ± 13.41 (S.E.M.) mg at 50 days after tumour transfer, compared with 40.80 ± 1.70 mg, total adrenal weight of control rats).
all extraction methods tested, 200 mmol HCl/l without boiling was selected on the basis of the maximum amount of ACTH and LPH/endorphin immunoreactivity (IR) extracted and maximum recovery of added synthetic peptide. Radioimmunoassay dilution curves obtained with tumour extracts were parallel to the respective standards with all RIA systems employed (results not shown), thus making it possible to quantify the amounts of the different POMC peptides present in the extracts. Tumour ACTH, α-MSH, β-LPH/endorphin and N-POMC content correlated well with tumour growth, being linear between day 15 and day 50 after inoculation (correlation coefficient, \( r > 0.90 \); results not shown). Mean ACTH content of tumours was \( 93.40 \pm 5.27 \) pmol/g by RIA and \( 65.52 \pm 3.49 \) pmol/g by bioassay; immunoreactive N-POMC, β-LPH/endorphin and α-MSH content were \( 99.03 \pm 4.87 \) pmol/g, \( 89.74 \pm 8.20 \) pmol/g and \( 2.83 \pm 0.13 \) pmol/g respectively (means ± S.E.M., \( n > 30 \)).

Figure 1a shows the ACTH and α-MSH RIA profiles of a Sephadex G-75 chromatogram of a normal (Buffalo) rat pars distalis extract. Four peaks of ACTH IR were detected. A peak of about 30,000 mol. wt coincided with the expected elution position of the precursor (POMC). A second peak of about 23,000 mol. wt probably accounts for the N-POMC/ACTH intermediate. A third peak of about 6500 mol. wt corresponded to the so-called 13K ACTH (Eipper & Mains, 1977) which represents glycosylated ACTH(1–39). Finally, a peak coinciding with synthetic ACTH(1–39) was detected. A peak which may be true α-MSH also eluted at the expected position for synthetic α-MSH. Two different pools of tumours and at least four individual tumours were analysed chromatographically. Both individual tumours and pools of tumours gave essentially the same chromatographic profiles with all RIA systems employed. Hence, a typical chromatogram corresponding to one of the pool of tumours analysed is shown in Fig. 1b for ACTH IR. A large IR-ACTH peak was located at the position of 20,000 mol. wt ACTH. A smaller peak of ACTH IR appeared at the position of ACTH (1–39) and a smaller sized IR-ACTH peak was detected, coinciding with synthetic ACTH (1–24). Neither a 30,000 mol. wt precursor peak nor a 6500 mol. wt peak could be detected in any of the tumour chromatograms. In the case of the smaller ACTH-like peak detected, all efforts were made to discard the possibility that this peak was the result of an artificial cleavage of larger ACTH peptides during extraction and/or chromatography. All the extraction procedures employed yielded approximately the same proportion of the smaller sized ACTH peptide. Moreover, the addition of \(^{125}\)I-labelled ACTH(1–39) to tumour fragments at the time of homogenization did not yield any labelled product with the elution characteristics of ACTH(1–24). The ACTH(1–24)-like peak also showed N-terminal ACTH IR but was devoid of C-terminal ACTH IR using an antiserum which did not cross-react with authentic ACTH(1–24) (results not shown). Chromatography of perfusion media from tumour cells gave essentially similar chromatographic profiles as tumour extracts, including the smaller IR-ACTH peak (results not shown). Both the 4500 mol. wt IR-ACTH peak and the 3000 mol. wt IR-ACTH peak displayed ACTH bioactivity when assayed in a rat adrenal cell system with similar relative biological potencies (bioassay to RIA ratios between 0.5 and 0.6 for both molecular forms). All IR-ACTH peaks gave dilution curves parallel to the standard curve with the ACTH assay employed. The α-MSH RIA revealed a single peak eluting at the position expected for synthetic α-MSH which accounted for less than 3% of the total ACTH IR detected in all ACTH peaks.

Figure 2 shows the chromatographic profiles of N-POMC IR of rat pars distalis extracts (Fig. 2a) and the pool of tumours also described in Fig. 1 (Fig. 2b). In pars distalis extracts, a peak with a molecular weight of about 30,000 could be seen, which probably represents POMC. Another peak was detected which also showed ACTH IR and could account for the 23,000 N-POMC/ACTH intermediate (Jackson, Salacinski, Hope & Lowry, 1983). Both precursor peaks gave slightly non-parallel dilution curves when compared with the hnPOMC(1–76) standard curve. Finally, two large closely eluting peaks were detected with sizes similar to N-POMC(1–95) and N-POMC(1–74) respectively. In the tumour chromatograms, the N-POMC RIA revealed no immunoreactivity at the position of the precursor, confirming the findings when the ACTH RIA was used. Instead, a peak of N-POMC IR was found at the position of the 20,000 mol. wt intermediate and, in agreement with the paras distalis chromatograms, tumour chromatograms displayed peaks corresponding to N-POMC(1–95) and N-POMC(1–74). No smaller IR-N-POMC peaks were detected in tumour or pars distalis extracts.

The C-terminal β-LPH RIA of Buffalo rat pars distalis chromatography (Fig. 3a) confirmed previous reports on other rat strains (Jackson & Lowry, 1980), namely the presence of a peak representing POMC, a major IR-endorphin peak corresponding to β-LPH and a smaller peak at the position of β-endorphin. Tumour extract chromatograms displayed unusual characteristics regarding LPH/endorphin IR (Fig. 3b). A small peak of about 20,000 mol. wt was detected which could not be ascribed to any known molecular species containing the LPH region of the precursor. A small peak at the position of β-LPH was detected, accounting for 13% of the total LPH/endorphin IR detected. Another peak eluted at the
position of \( \beta \)-endorphin which accounted for about 17% of LPH/endorphin IR. Finally, a large peak of low molecular weight was detected with an apparent mol. wt of about 2000 which could be ascribed to \( \alpha \)- or \( \gamma \)-endorphin or another fragment of the C-terminal region of \( \beta \)-LPH. Here again, artifacts could modify the actual distribution of LPH/endorphin IR along the chromatograms although extraction conditions were optimized to avoid degradation of \( \beta \)-LPH and related peptides (Ueda, Takeuchi, Abe et al. 1980). Recovery of the different immunoreactive peaks after chromatography fluctuated between 85 and 95% with all RIA systems employed.

Affinity chromatography on Concanavalin A-agarose of the different peaks obtained after G-75 chromotography of tumour extracts was performed in order to establish the presence of glycosylated POMC peptides. As expected, the peaks on pars distalis chromatograms corresponding to 30 000 mol. wt, 23 000 mol. wt and 6500 mol. wt ACTH IR, and 30 000 mol. wt and 23 000 mol. wt N-POMC(1–95) and N-POMC(1–74) IR, all partially bound to Concanavalin A-agarose columns and were specifically eluted with methylglycoside. Tumour peaks corresponding to 20 000 mol. wt (both ACTH and N-POMC IR) and N-POMC(1–95) and N-POMC(1–74) were also retained in Concanavalin A-agarose columns and specifically eluted with methylglycoside. The proportion of unretained versus retained immunoreactivity of analogous peaks did not vary significantly between rat pars distalis and tumour material. The percentage
of IR material specifically bound by the lectin varied between 27 and 33% for the different peaks assayed. All other IR-POMC peaks from Sephadex chromatograms and non-glycosylated standard peptides were not specifically retained by the Concanavalin A–agarose columns (results not shown).

DISCUSSION

More than 20 years ago tumour 7315a was reported to produce ACTH (Bates et al. 1966), but since then no attempts have been made to characterize the ACTH peptides it produces, and only one paper mentions different molecular forms of β-LPH/endorphin-like IR being resolved by gel filtration chromatography (Panerai et al. 1980). Moreover, the content of ACTH and other POMC peptides in the tumour has not been conclusively determined; the original study of Bates et al. (1966) only used an in-vivo ACTH bioassay. This tumour and other M1T tumours (i.e. M1TF4) have been employed extensively to cause hyperprolactinæmia in their host (MacLeod et al. 1966; Lamberts, Uitterlinden, Bons et al. 1984), and to a lesser extent, augment levels of ACTH in blood (Bates et al. 1966). The effects of high levels of ACTH in tumour-bearing animals have been repeatedly described as presenting dramatic morphological changes (Bates et al. 1966; MacLeod & Lehmeyer, 1973).

In this context, we have found it interesting to study the entire spectrum of POMC peptides produced by the tumour, taking into account the wide effects that have been ascribed to POMC-derived peptides on adrenal growth and function (Pedersen et al. 1980; Al-Dujaili et al. 1981; Matsuoka et al. 1981; Vinson, Whitehouse, Dell et al. 1981; Estivariz et al. 1982; Lowry et al. 1983). In addition it was interesting to observe whether any differential processing of POMC occurred in the 7315a tumour compared with normal rat tissue or other tumours, which could give additional information about the mechanisms involved in POMC processing. For this purpose we have employed gel filtration chromatography coupled to different RIA systems which have been used to characterize POMC peptides (Jackson & Lowry, 1980; Hope & Lowry, 1981; Jackson et al. 1983; Estivariz & Iturria, 1985). The values originally reported for the ACTH content of tumour tissue are difficult to compare with our own results because of the type of assay employed, but our data do not seem to correlate well with the earlier values. As expected, tumour content of the different POMC peptides showed good correlation with tumour mass. Hormone concentration did not vary significantly with the size of the tumour. Absolute amounts of ACTH, β-LPH/endorphin and N-POMC detected in tumour homogenates were roughly equimolar, while α-MSH content of the tumour was about 30 times less than all the other POMC peptides in molar equivalents.

Chromatography of pars distalis homogenates of Buffalo rats did not show any significant differences with regard to POMC peptides from those of other rat strains previously investigated (Jackson & Lowry, 1980; Jackson et al. 1983), apart from minor changes in the relative proportions of 30 000 mol. wt precursor and glycosylated ACTH, which were higher in Buffalo than in Wistar rats.

The chromatographic profiles of tumour ACTH IR gave an unusual pattern of peaks. There was a large peak of ACTH IR which eluted just after the postulated rat pars distalis 23 000 mol. wt intermediate, which could represent a smaller N-POMC/ACTH
intermediate in the tumour. This difference in size could be explained by a lesser degree of glycosylation of the tumour intermediate. Only a smaller amount of immunoactivity was detected at the position of ACTH (1–39), in contrast to the relatively large proportion of this peptide formed in pars distalis chromatograms. Conversely, a significant amount of ACTH IR was located at the elution position of ACTH (1–24). This IR peak (as well as the other POMC-derived IR peaks) was also detected after chromatography of tumour cell incubation media which was collected in conditions in which artifactual cleavage of secreted peptides were minimized (Gillies et al. 1980). Moreover, radiolabelled ACTH (1–39) added to tumour extracts did not yield any peptide resembling the one detected in the tumours at the conditions of extraction selected. According to the high bioactivity to immunoactivity ratio displayed by this peptide, it should have the entire N-terminal sequence of ACTH (i.e. ACTH (1–18)). On the other hand, a peptide considerably smaller than ACTH (1–24) would be expected to be generated during the cleavage to produce α-MSH and corticotrophin-like intermediate lobe peptide (Scott, Ratcliffe, Rees et al. 1973). If these assumptions are correct, an unusual cleavage site in the ACTH sequence may have arisen, caused by a mutational change in the tumour cell DNA which could generate such a peptide. Further characterization of this peptide is in progress.

As in the pars distalis chromatogram, a peak with both chromatographic and radioimmunological characteristics of true α-MSH was detected in tumour chromatograms, as already described in other POMC-producing tumours (Mains & Eipper, 1981; Shibasaki, Masui, Sato et al. 1981).

In the case of N-POMC peptides, two IR peaks, although not well resolved, were detected at elution positions compatible with N-POMC (1–95) and N-POMC (1–74) respectively, in both pars distalis and tumour extracts. A large peak, which also coincided with an IR-ACTH peak, may also account for the 20 000 mol. wt intermediate, which contains both N-POMC and ACTH sequences. Interestingly, there were no smaller N-POMC peptides such as those found in rat pars intermedia (Jackson et al. 1983).

β-Endorphin-like IR of tumour extracts also showed remarkable differences compared with pars distalis chromatograms. Only a minor peak, probably accounting for rat β-LPH which is about 20 amino acids smaller than hβ-LPH, was detected. In addition a small amount of endorphin IR was detected in a peak larger than hβ-LPH which cannot account for any known POMC fragment containing LPH/endorphin. Most of the β-endorphin IR eluted close to the bed volume of the chromatograms. Again, we could not ascribe this peak to an artifactual cleavage of larger peptides after homogenization. It could account, perhaps, for α- or γ-endorphin which has previously been found in rat pars intermedia (Vaudry, Pelletier, Guy et al. 1980) and human pituitary gland (Ueda et al. 1980).

In contrast with that found in pars distalis extracts, no peak was detected in the position of the precursor POMC with any of the RIA systems employed. Most POMC-secreting tumours so far studied have been shown to contain POMC in various proportions (Bertagna, Nicholson, Sorensen et al. 1978; Mains & Eipper, 1981; Hale, Besser & Rees, 1986). The complete absence of non-processed precursor in 7315a tumour resembles the situation in normal (but not tumoral) human pituitary which stores only minimal amounts of POMC (Lowry, Hope & Silman, 1976; Orth & Nicholson, 1977).

We have not been able to detect γ-LPH-like peptides or γ3-MSH in tumours because of the lack of appropriate cross-reaction of tumour material with the RIA systems employed.

With respect to glycosylation of POMC-derived peptides from the tumour, we used the Concanavalin A method previously employed to demonstrate POMC-glycopeptides in normal pituitary (Jackson & Lowry, 1980) or POMC-producing tumours (Eipper, Mains & Guenzi, 1976; Orth & Nicholson, 1977). The POMC-derived peptides from the tumour did not show any significant change from that observed in rat pars distalis material (this study; Jackson & Lowry, 1980). The proportion of bound to excluded IR from the mouse pituitary tumour cell line AT T20/D-16v (Eipper & Mains, 1977); however, it is interesting to note that normal and tumorous human pituitaries also lack 6500 mol. wt. ACTH (Allen, Orwell, Kendall et al. 1980).

As mentioned above, the presence in the tumour of POMC peptides other than ACTH (1–39) which are actually secreted in vitro and probably circulate at high levels in tumour-bearing animals, raises the interesting question of whether the changes observed in the adrenal glands of those animals are due to ACTH (1–39) alone or in combination with other POMC-derived peptides. In this respect, recent reports from this and another laboratory (Estivariz et al. 1982; Lowry et al. 1983; Lowry, Estivariz, Silas et al. 1984) have implicated N-POMC peptides in adrenal growth. Since the adrenal glands of the tumour-bearing rats showed considerable hypertrophy, it is tempting to speculate that N-POMC peptides produced by the tumour may contribute to the hypertrophy observed in the adrenal glands of the host.
N-POMC(1-76) and N-POMC(51-77) (γ3-MSH) have been shown to potentiate the action of ACTH on the adrenal by altering the activity of enzymes involved in corticosteroid synthesis (Pedersen et al. 1980; Al-Dujaili et al. 1981). Accordingly the increased amount of N-POMC peptides and ACTH (including forms other than ACTH(1-39)) produced by the tumour may also be responsible for the structural and biochemical changes observed in the adrenal glands of tumour-bearing rats, such as mitochondrial ultrastructural changes and impaired corticosterone to 11-deoxycorticosterone ratios which, according to some authors, cannot be ascribed to augmented levels of ACTH alone (Brownie, Nickerson, Joziwjak et al. 1970; Pedersen & Brownie, 1980).

In conclusion, any future studies on the effect of 7315a tumour on the adrenal gland of a tumour host will have to take into account all the peptides it produces. In addition, the peculiarities observed in the processing of POMC in this tumour may render this tissue an interesting model in which to elucidate the processing of POMC.

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