β-Endorphin and ACTH related peptides in primary cultures of rat anterior pituitary cells: evidence for different intracellular pools

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Acid extracts of rat anterior pituitary cells and cell-derived culture media were shown to contain three forms of β-endorphin immunoreactive peptides, corresponding in molecular size to the prohormone pro-opiomelanocortin (POMC), β-lipotropin and 3.5 kDa β-endorphin, and essentially two forms of adrenocorticotropin (ACTH) immunoreactivity, representing a 20 kDa intermediate fragment and 4.5 kDa ACTH. Under basal conditions the intracellular peptides contained a high proportion of the bioactive forms of β-endorphin and ACTH whereas the extracellular peptides contained a higher proportion of the inactive precursors. When the cells were incubated for 3 h in the presence of 10^{-8} M CRF, the levels of intracellular β-endorphin and ACTH immunoreactivity were reduced by 15–30% and there was a 4–5-fold increase in the level of the secreted peptides; furthermore, unlike the peptides released under basal conditions, the peptides secreted under the influence of CRF contained much higher proportions of 4.5 kDa ACTH and 3.5 kDa β-endorphin, reflecting the intracellular patterns of these peptides. Similar results were obtained when secretion was stimulated by 10^{-7} M epinephrine, which produced a 2-fold increase in peptide release. In the presence of 10^{-6} M dexamethasone the basal secretion of ACTH and β-endorphin related peptides, and the intracellular levels of these peptides, remained unaltered. The results point to the existence of different intracellular compartments from which peptides at different states of maturation can be released selectively.

β-Endorphin *ACTH* *Pituitary cell culture* *Processing* *CRF* *Epinephrine*

1. INTRODUCTION

Pro-opiomelanocortin (POMC), the precursor of the bioactive peptides adrenocorticotropin (ACTH), β-endorphin and α-melanocyte-stimulating hormone (α-MSH), is synthesised in the brain where the end products appear to serve as neurotransmitters [1–3] and in the pituitary where the peptides are stored preparatory to release as hormones [4,5]. The extent of conversion of POMC to its bioactive fragments, however, varies from tissue to tissue. In rat pars intermedia, for example, processing of the prohormone proceeds nearly to completion, generating the acetylated forms of α-MSH and the shortened forms of β-endorphin as the major peptides [6–9]; in contrast, proteolysis of POMC in the pars distalis is less complete, yielding predominantly ACTH and β-lipotropin [5,8,10]. This 'tissue specific' processing of POMC is also evident in regions of brain; desacetyl α-MSH and β-endorphin are the main products in rat hypothalamus [7,11] whereas acetylated and truncated forms of these peptides are present in regions distal from the hypothalamus [7,11,12]. Thus, variations in the processing of the POMC prohormone serve to generate different bioactivities in different tissues.

An interesting aspect of POMC processing is that the processing reactions can generate prohormone fragments that are highly active together with related peptides that are virtually inactive. In the pars intermedia, for example, β-endorphin

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which is a potent analgesic agent [13] can be processed to generate acetylated derivatives which are essentially devoid of analgesic activity [14,15]; and these inactivated peptides occur in company with acetylated forms of α-MSH which are highly potent [16]. In this way a complementary relationship may exist between different biological activities generated from a common prohormone through the potentiation of one activity and attenuation of another.

Recently we reported that the processing of POMC in rat pars intermedia is sensitive to the presence of dopaminergic agonists which not only inhibit the rate of secretion but also alter the pattern of peptides secreted [17]. It appears that the processing of POMC in the pars intermedia is linked to secretion: in particular when the cells are in a dynamic state of secretion, processing of the POMC prohormone is directed towards the formation of bioactive products whereas under conditions of basal secretion it is principally the inactive forms that are released. Here we report that under conditions of stimulated secretion the principal forms of ACTH and β-endorphin released from the pars distalis of the pituitary are the bioactive peptides, 3.5 kDa β-endorphin and 4.5 kDa ACTH.

2. MATERIALS AND METHODS

2.1. *Preparation of primary cultures of rat anterior pituitary cells*

Monolayers of rat anterior pituitary cells were prepared by the method of Kirkland and Ellison [18]. For experimental treatments the cells were incubated for 3 or 6 h in the presence and absence of 10^{-8} M ovine corticotrophin-releasing factor (RIA UK Ltd), 10^{-7} M epinephrine bitartrate (Sigma) and 10^{-6} M dexamethasone sodium phosphate (kindly donated by Merck, Sharp and Dohme); the anti-oxidant ascorbic acid (final concentration 100 μg/ml) was included.

2.2. *Extraction of β-endorphin and ACTH related peptides*

Cell monolayers were harvested by scraping the cells into ice-cold acetic acid (20%, v/v) with the aid of a silicon rubber policeman. The cell extracts were rapidly homogenised at 4°C, centrifuged for 20 min, 40000 × g at 4°C and the supernatants applied directly to gel exclusion chromatography or lyophilised for storage. Cell-exposed culture medium was acidified with hydrochloric acid (0.1 ml of 1 M HCl:5 ml culture medium), lyophilized and reconstituted in 20% acetic acid prior to chromatography.

2.3. *Chromatography and radioimmunoassay*

β-Endorphin and ACTH related peptides were resolved by gel exclusion and ion-exchange chromatography using respectively Sephadex G-75 and SP-Sephadex C-25 (pyridinium form) as in [19]. ACTH measurements were made by radioimmunoassay using an antiserum raised against porcine ACTH coupled to thyroglobulin; its specificity was directed against the mid-region of ACTH. The procedure employed was similar to that used for the β-endorphin assay [11]. The specificity of the β-endorphin antiserum was directed towards the carboxyl terminus. Both antisera were used at a final dilution of 1:16000.

3. RESULTS

In 2 cultures of anterior pituitary cells which were incubated with 10^{-8} M CRF for 3 or 6 h, there was a substantial increase in the level of secreted β-endorphin and ACTH related peptides (fig.1), concomitant with a fall in the intracellular levels. In addition the level of the total immunoreactive peptides (intracellular and extracellular) related to β-endorphin and ACTH was higher (30% above control) for the CRF-treated cultures than for the untreated cells. Similar findings were observed with 10^{-7} M epinephrine as secretagogue when a 2-fold increase in secretion occurred; this stimulated secretion was again accompanied by a decrease in the level of the intracellular peptides. When the cultures were incubated in the presence of 10^{-6} M dexamethasone, the basal secretion of ACTH and β-endorphin related peptides and the intracellular levels of these peptides were essentially unaltered.

Acid extracts of cells and cell-derived culture media were subjected to gel-exclusion chromatography, showing different patterns of both β-endorphin and ACTH immunoreactivity. The intracellular and extracellular extracts contained 3 forms of β-endorphin immunoreactivity, corresponding to POMC prohormone, β-lipotropin

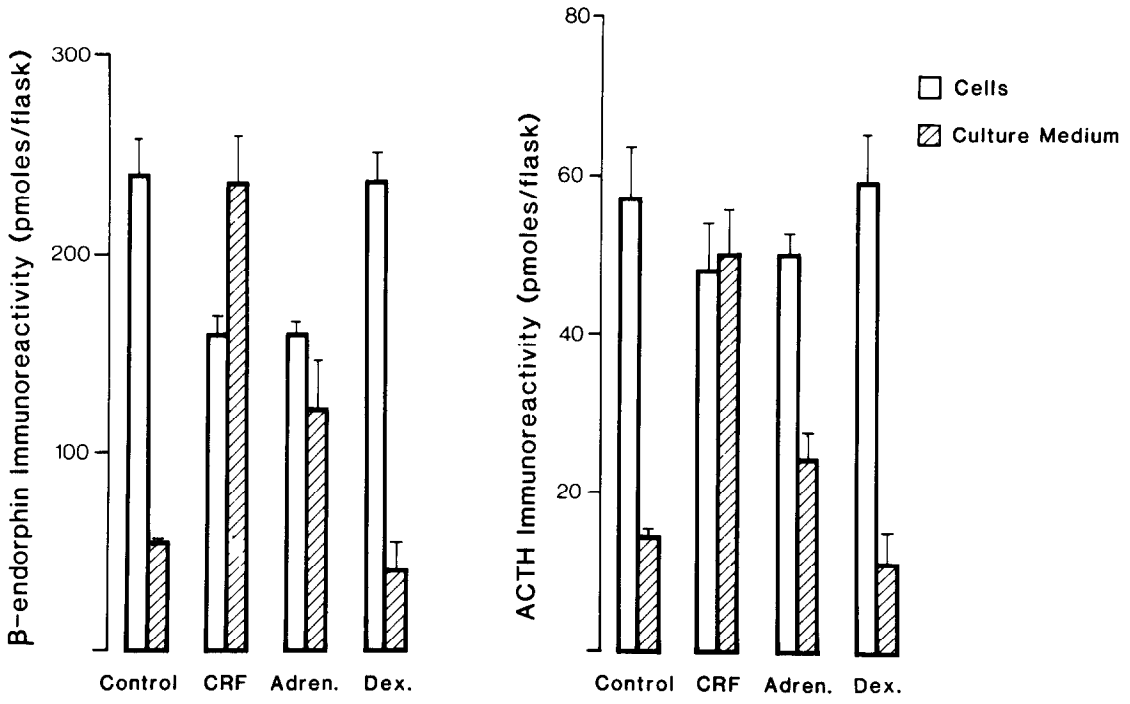

Fig.1. The effects of corticotrophin-releasing factor, adrenalin (epinephrine) and dexamethasone on intracellular and extracellular immunoreactive β-endorphin and ACTH in rat primary anterior pituitary cultures.

and 3.5 kDa β-endorphin, and 2 peaks of ACTH immunoreactivity, one eluting in the position of 4.5 kDa ACTH and the other in a position corresponding to an apparent molecular mass of 20–25 kDa (figs 2–4). Further purification by ion-exchange chromatography showed that the β-endorphin sized material resolved by gel exclusion consisted of a single chromatographic component which co-eluted with ^{125}I-porcine β-endorphin 1–31; there was no evidence for the presence of a significant amount of the 26- and 27-residue forms of β-endorphin (fig.5).

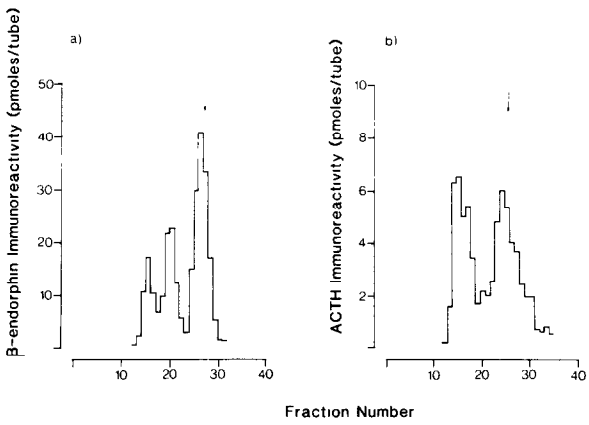

Fig.2. Gel-exclusion resolution of (a) β-endorphin related peptides and (b) ACTH related peptides, in extracts of rat anterior pituitary cells. The arrow indicates the elution position of ^{125}I-β-endorphin 1–31.

Examination of the gel exclusion profiles of β-endorphin and ACTH peptides in extracts of control anterior pituitary cells (fig.2) showed that 3.5 kDa β-endorphin and 4.5 kDa ACTH represented approx. 50% of the total respective immunoactivities. In the culture medium the level of these peptides was considerably reduced (figs 3 and 4); the predominant peptides secreted under basal conditions were the larger, precursor forms of β-endorphin and ACTH. In contrast, in the presence of CRF the levels of 3.5 kDa β-endorphin and 4.5 kDa ACTH secreted into the culture medium were increased relative to the total β-endorphin or total ACTH immunoreactivity (figs 3 and 4); the patterns of peptides secreted from these cells resembled the intracellular patterns characteristic of both the control cells and the

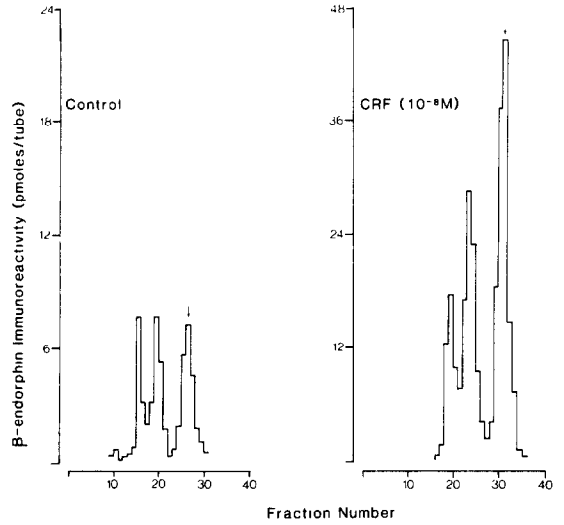

Fig.3. Gel-exclusion resolution of secreted β-endorphin related peptides from control and CRF-treated rat anterior pituitary cells. The arrow indicates the elution position of ^{125}I-β-endorphin 1–31.

CRF-treated cells. This enhancement of 3.5 kDa β-endorphin and 4.5 kDa ACTH in the peptides secreted under the influence of CRF was also seen in the epinephrine-treated cultures, though the effects were less marked. In the presence of 10^{-6} M dexamethasone the profiles of intracellular and extracellular β-endorphin and intracellular and extracellular ACTH did not differ from the patterns exhibited by the control cells.

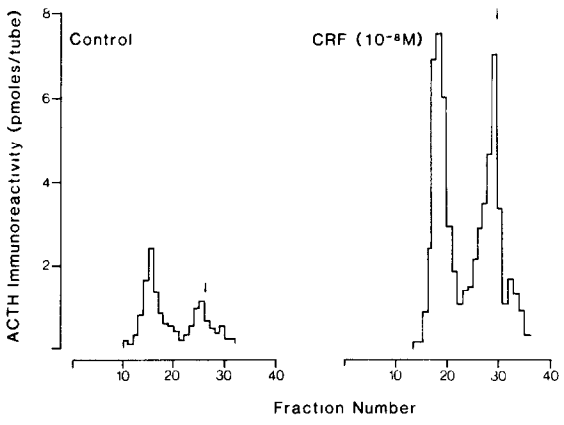

Fig.4. Gel exclusion resolution of secreted ACTH-related peptides from control and CRF-treated rat anterior pituitary cells. The arrow indicates the elution position of ^{125}I-β-endorphin 1–31.

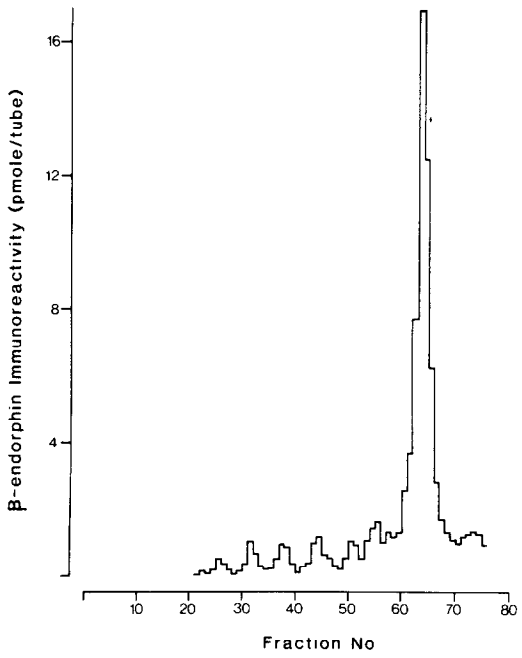

Fig.5. Ion-exchange chromatographic resolution of β-endorphin size peptides secreted from rat control anterior pituitary cells (showing that β-endorphin 1–31 is the major peptide). The arrows from left to right indicate the elution positions of radio-labelled ^{125}I-reference markers: α-*N*-acetyl β-endorphin 1–27, β-endorphin 1–27, α-*N*-acetyl β-endorphin 1–31 and β-endorphin 1–31.

It should be noted that in these experiments complete resolution of 13 kDa ACTH (glycosylated ACTH) from 20 kDa ACTH was not achieved with the methods employed; however, the glycosylated peptide has been reported to be only a minor component of the total ACTH present in rat anterior pituitary [6].

4. DISCUSSION

The finding that anterior pituitary cells under basal conditions secrete preferentially higher molecular mass peptides while the peptides secreted under stimulus are mainly the bioactive forms suggests that distinct pools of POMC related peptides may exist, one destined for secretion and another for storage. This is consistent with the data of Gumbiner and Kelly [20] who have proposed that 2 intracellular transport pathways are operative in the AtT-20 pituitary cell line.

Another possibility which could account for the different intracellular and extracellular patterns is that the secreted peptides might originate from more than one cell type: under basal, non-stimulated conditions secretion would appear to take place from cells containing immature precursor peptides whereas under stimulated conditions of secretion cells containing principally the bioactive peptides could be called into the secretory pathway. In this context it should be noted that Deftos and Catherwood [21], using immunoperoxidase techniques, obtained evidence for the existence of different populations of POMC-containing cells in the anterior pituitary, one type elaborating both ACTH-related and β-endorphin related peptides and another only ACTH-related peptides; the different cell types, however, could also reflect various states of maturity of the corticotrophs. Nevertheless, the existence of either different cell types or different peptide pools would offer an explanation for the patterns of peptides secreted in the presence of the secretagogues, CRF and epinephrine.

During CRF stimulated secretion the total ACTH and β-endorphin related peptides (stored + released) exceeded the levels of these peptides in the control cells, suggesting that de novo synthesis of POMC was increased or that degradation of the biosynthetic products was inhibited. Increased synthesis is less likely since the cells were exposed to CRF for no more than 6 h. It has been shown previously that the intracellular levels of ACTH in vitro [22] and POMC mRNA in vivo [23] increase only after chronic stimulation with a secretagogue over a period of several days. The postulation of decreased rates of intracellular degradation is more attractive as it would tend to restore the bioactive peptides lost to the cell during secretion. In this context it has been demonstrated by pulse chase studies in AtT-20 cells that intracellular degradation of β-endorphin is strongly inhibited when the cells are exposed to CRF (S. Cockle, personal communication).

It should be noted that the mode of action of CRF and epinephrine is not fully understood. Evidence has been obtained that CRF stimulated secretion is mediated via specific receptors and is cAMP dependent [24]; in contrast epinephrine appears to act through a different pathway [25]. Furthermore the action of CRF can be potentiated by synergism with other hormones such as vasopressin, oxytocin and epinephrine [25,26]. Since the intracellular and extracellular peptide patterns are similar under conditions of dynamic secretion, it would appear that the processing reactions involved in the cleavage of POMC are not directly affected by the secretion stimulus. It might be anticipated that during rapid secretion the degree of processing would be reduced because of diminished exposure to the processing enzymes. On the contrary, the gel-exclusion profiles obtained in our experiments suggest that during the secretory period the rate of processing is in fact increased, though the increased processing may be counterbalanced in part by a decrease related to augmented secretion. While further study will be necessary to clarify the specific actions of CRF and related secretagogues, the present results indicate that both inhibition of degradation and stimulation of processing take place during secretion.

That β-endorphin is co-released with ACTH from the anterior pituitary suggests that this opiate peptide may play a supportive role in modulating biological responses under stress, possibly by regulating the mobility of the gastrointestinal tract [27] or in the regulation of chromaffin cell activity [28]. In the present experiments the predominance of total β-endorphin immunoreactivity over total ACTH immunoreactivity (molar ratio 4:1) during both basal and the early stages of stimulated secretion is surprising since ACTH and β-endorphin related peptides are generated from a common prohormone and would be expected to occur in stoichiometric amounts. It is of interest that similar findings have been reported in a study of the stored and released peptides of AtT-20 cells [29]. It is, of course, possible that the precursor forms of ACTH are less reactive than 4.5 kDa ACTH in the ACTH radioimmunoassay whereas POMC, β-lipotropin and β-endorphin appear to be equally reactive in the β-endorphin RIA. Alternatively, the imbalance could be due to differential rates of degradation or selective secretion of the ACTH and β-endorphin related peptides.

The main finding of this study is that the patterns of peptides secreted from cultured anterior pituitary cells do not always correspond to the intracellular patterns. Thus the peptides secreted under basal conditions would appear to originate from different intracellular pools, or from dif-

ferent cell types, than the peptides that are secreted under the influence of secretagogues. Our findings are in agreement with the results of Allan et al. [30], using mouse anterior pituitary cells, and those of Mains and Eipper [31] employing AtT-20 cells. Furthermore, the suggestion that the cleavage of POMC to lipotropin occurs earlier and in a different compartment from that where the cleavage of β-lipotropin to β-endorphin takes place [32] is compatible with the existence of different pools within a cell. The different pools may respond to different secretory signals.

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