Regulation of bioactive β-endorphin processing in rat pars intermedia

J. Ham and D.G. Smyth

National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, England

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Acid extracts of rat pituitary neuro-intermediate lobes have been shown by ion-exchange chromatography and radio-immunoassay to contain predominantly the inactive derivatives of β-endorphin, α,*N*-acetyl β-endorphin 1–27 and α,*N*-acetyl β-endorphin 1–26; the biologically active form, β-endorphin 1–31, is a minor component. In contrast, it was found that β-endorphin generated in neuro-intermediate lobe cells in monolayer culture was less processed: the principal peptides related to bioactive β-endorphin 1–31. When the cultured cells were incubated in the presence of 10^{-5} M dopamine or 10^{-6} M α-ergocryptine there was a marked increase in the degree of proteolysis and acetylation: the processing pattern reverted to that characteristic of the neuro-intermediate lobe in situ, with α-*N*-acetyl β-endorphin 1–26 and α,*N*-acetyl β-endorphin 1–27 as the prominent peptides. The results demonstrate that dopaminergic agents can influence the processing of β-endorphin-related peptides in rat pars intermedia, indicating a new level at which the bioactivity may be regulated.

Endorphin *Neuro-intermediate lobe* *Processing* *Bioactivity* *Dopaminergic agent* *Acetylation*

1. INTRODUCTION

Six forms of β-endorphin are known to be present in the pars intermedia lobe of rat and porcine pituitary [1,2]. These peptides include the parent molecule, β-endorphin 1–31, its shortened forms generated by specific proteolysis, β-endorphin 1–27 and β-endorphin 1–26, and the corresponding α,N-acetylated derivatives. The physiological significance of this array of peptides is not known, but pharmacological studies have shown that β-endorphin 1–31 has potent analgesic properties [3,4] and is highly active in the rat vas deferens bioassay [5]; the 26- and 27-residue peptides are active in the rat vas deferens but retain little analgesic activity; the acetylated peptides have no opiate properties [6,7]. The majority of the β-endorphin-related peptides in the pars intermedia are the inactive forms [1] whereas in the pars distalis and the medial basal hypothalamus [8] β-endorphin 1–31 predominates. Thus, β-endorphin undergoes inactivation in varying degrees and it could be of physiological and clinical importance to understand the controlling factors that influence its processing.

The release of β-endorphin and α-melanotropin (α-MSH) from the pars intermedia in vivo is inhibited by dopaminergic innervation [9]. This level of control can be demonstrated in vitro by the addition of exogenous dopaminergic agents to neuro-intermediate lobe cells (pars nervosa + pars intermedia) in culture [10–12]. A possible relationship between processing and secretion, however, has not been explored. To investigate whether the pattern of β-endorphin-related peptides in the pars intermedia reflects the dynamic state of the cells, with respect to secretion, we have used monolayer culture as a model system in which the restraint im-

Abbreviation: POMC, pro-opiomelanocortin

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posed by dopamine innervation is not present and have compared the profile of peptides present to that which is seen in the same tissue in situ.

2. MATERIALS AND METHODS

2.1. *Preparation of primary cultures of neuro-intermediate lobe cells*

Male Sprague Dawley rats (200–250 g) were killed with a lethal dose (120 mg) of sodium pentobarbitone. After decapitation, the pituitary gland was removed and separated aseptically into the neuro-intermediate and anterior lobes. The neuro-intermediate lobes were frozen and stored at −70°C or used to prepare primary cultures.

Tissues were cut into approximately 1 mm^3 pieces and treated with 2 ml of 0.125% trypsin (Worthington) at 37°C for 15 min with occasional agitation. The tryptic wash was discarded and the enzymic treatment repeated. The residual tissue was then treated for 20 min at 37°C with 2 ml of 1 mg/ml collagenase (Boehringer, Mannheim). Separation into single cells was performed manually by drawing the tissue pieces repeatedly through a fine glass pipette. The enzymic solution was inactivated and separated from the cells by centrifugation. The yield of viable cells was approximately 2×10^5 per neuro-intermediate lobe. Cells were suspended in 90% Dulbecco's Eagles medium (Hepes buffered) supplemented with 10% foetal calf serum, 100 μg/ml kanamycin and 2.5 μg/ml amphotericin B, and plated in 25 cm^2 tissue culture flasks at a density of 5×10^5 cells per flask. After an initial period (2–3 days) of attachment of the cells to the flask, the culture medium was replaced at 2-day intervals. For experimental purposes the confluent monolayers were incubated for 2 h in the presence or absence of 10^{-6} M 2-bromo-α-ergocryptine methane sulphonate or 10^{-5} M dopamine hydrochloride. In these experiments the antioxidant ascorbic acid (final concentration 100 μg/ml) was included.

2.2. *Extraction of β-endorphin related peptides*

Tissues from 5 animals were disrupted for 3–4 min in a hand-held teflon homogeniser with 2 ml ice-cold 2 M acetic acid. ^{125}I-labelled β-endorphin marker peptides (10 000 cpm) were routinely added to the extraction medium. The extracts were centrifuged for 20 min at 20 000 rpm, 4°C, using a Beckman J2-21 centrifuge and aliquots of the supernatant were submitted to gel exclusion chromatography.

Cell monolayers were harvested by scraping the cells into ice-cold acetic acid (2 M) with a silicon rubber policeman. The cell extracts were rapidly homogenised, centrifuged and the supernatants applied directly to gel exclusion chromatography or lyophilised for storage.

2.3. *Chromatography*

β-Endorphin-related peptides were separated on a 100×1 cm column of Sephadex G-75 (bead size 40–120 μm) equilibrated with 50% (v/v) acetic acid and 2-ml fractions were collected. The fractions corresponding to the elution position of the ^{125}I-labelled β-endorphin marker peptides were pooled and applied to a column (60×0.6 cm) of SP-Sephadex C-25 (pyridinium form). Ion-exchange chromatography was performed in 50% acetic acid with a linear gradient to 1 M pyridine in 50% acetic acid, mixer volume 100 ml; 2-ml fractions were collected [8].

2.4. *Radioimmunoassay*

Identification and quantitation of β-endorphin-related peptides were performed by radioimmunoassay and correspondence with the labelled marker peptides. Appropriate aliquots from gel filtration or ion-exchange chromatography were dried in vacuo at 20°C and reconstituted in radioimmunoassay buffer. The titre for β-endorphin antisera (with COOH terminal specificity) was 1:16 000. The incubation time was 16 h at 4°C and separation of the free from bound antigen was performed using dextran-charcoal. In all cases quantitative estimations were based on immunoreactive displacement relative to β-endorphin 1–31. The detailed protocol for the assay procedure has been described previously [13].

3. RESULTS

Virtually all of the β-endorphin immunoreactive peptides extracted from rat neuro-intermediate lobes co-eluted with [^{125}I]β-endorphin 1–31 during molecular size separation on Sephadex G-75; very little β-lipotropin size material was detectable. When the β-endorphin fraction was chromatographed on SP-Sephadex C-25, 6 peaks of im-

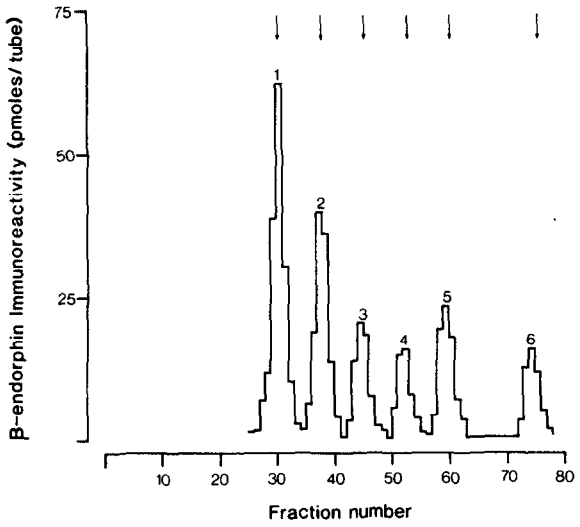

Fig. 1. Ion-exchange chromatography of β-endorphin-related peptides extracted from rat neuro-intermediate lobes in situ. The eluted fractions were determined by radioimmunoassay with a β-endorphin antiserum. The arrows (left to right) correspond to the elution positions of radio-labelled ^{125}I markers: α,*N*-acetyl β-endorphin 1-26, β-endorphin 1-26, α,*N*-acetyl β-endorphin 1-27, β-endorphin 1-27, α,*N*-acetyl β-endorphin 1-31 and β-endorphin 1-31.

munoreactivity were resolved (fig. 1). The prominent peptides were α,*N*-acetyl β-endorphin 1-26 and α,*N*-acetyl β-endorphin 1-27 (peaks 1 and 2 respectively) and β-endorphin 1-31 (peak 6) was a minor component. The extent of acetylation of each peptide relative to the corresponding NH_2-form increased in the order: β-endorphin 1-26>β-endorphin 1-27>β-endorphin 1-31. The degrees of proteolysis (sum of peaks 1-3) and of α,*N*-acetylation (sum of peaks 1,2 and 5) were >67 and >69% of the total β-endorphin, respectively.

The β-endorphin-related peptides in extracts of neuro-intermediate lobes in monolayer culture (3 and 10 days) exhibited significantly reduced degrees of proteolysis (<50%) and acetylation (<50%) (fig. 2a); the principal component was β-endorphin 1-31. However, as with the tissue in situ, the degree of acetylation was greatest with the shorter peptides.

When the cultures were incubated for 2 h in the presence of 2-bromo-α-ergocryptine methane sulphonate (10^{-6} M) or dopamine hydrochloride (10^{-5} M), there was an inhibition of secretion of β-

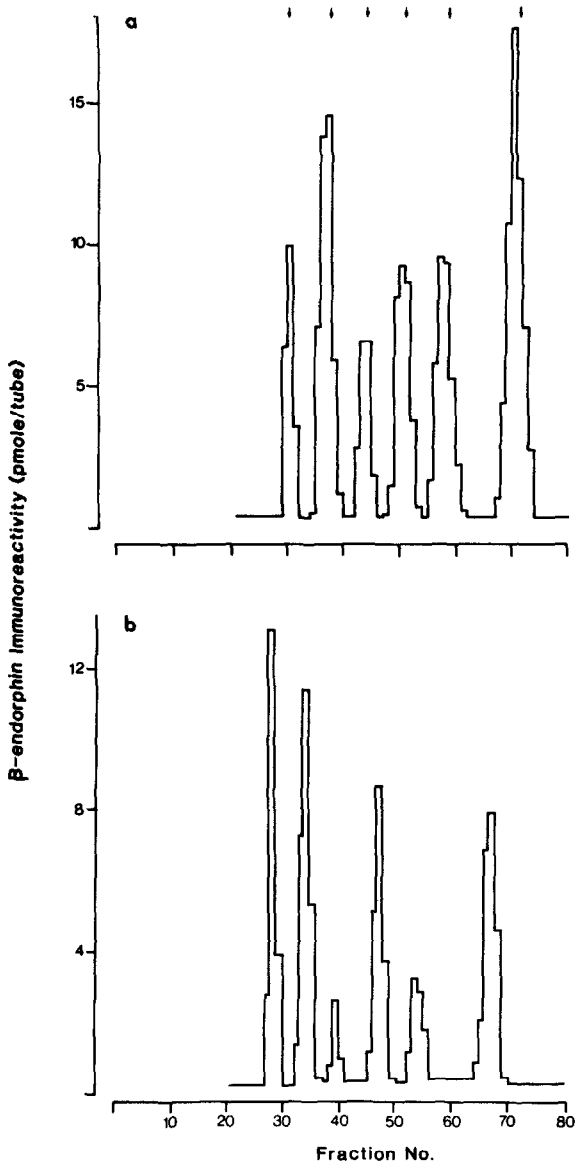

Fig. 2. Ion-exchange chromatography of β-endorphin-related peptides extracted from rat neuro-intermediate lobes in monolayer culture (10 days). (a) Control and (b) incubated in 2-bromo-α-ergocryptine methane sulphonate (10^{-6} M) for 2 h. The marker peptides eluted in the positions indicated by arrows, as in fig. 1.

endorphin-related peptides into the culture medium, accompanied by a fall (<75% of the control) in the cellular content of total β-endorphin. In the presence of the dopaminergic agents the relative levels of intracellular β-lipotropin and β-

endorphin were unaltered but the relative proportions of the β-endorphin-related peptides underwent a marked change: chromatography showed that α,N-acetyl β-endorphin 1–26 and α,N-acetyl β-endorphin 1–27 were the most prominent peptides (fig. 2b). The degree of both proteolysis and acetylation increased to >65%, compared with <50% in the control cells. This pattern of β-endorphin-related peptides in the dopamine- or ergocryptine-treated cultures approximated to that observed in the extracts of neuro-intermediate lobes in situ.

4. DISCUSSION

In the homeostatic state, secretion of peptides from the pars intermedia is under the inhibitory control of dopamine neurons arising from the medial basal hypothalamus. The finding that β-endorphin-related peptides in the pars intermedia in situ are mainly the inactive forms, as assessed by receptor binding [14,15] and analgesic properties [6,7], suggests that β-endorphin generated in the pars intermedia fulfills only a minor role while the processed forms may be non-functional. In monolayer culture, on the other hand, the inhibitory restraint on secretion is absent and the cells can be considered to be in the secretory mode. The pattern of β-endorphin-related peptides in these cells showed notably reduced degrees of proteolysis and acetylation, reflecting an apparent need for bioactive β-endorphin. To adapt to the increased rate of secretion, it is possible that the intracellular peptides undergo a more rapid turnover and the reduced proteolysis and acetylation observed could be attributed to a decreased exposure to the specific processing enzymes or to a reduction in the rate of biosynthesis of the enzymes.

The addition of dopaminergic agents to neuro-intermediate cells in culture led, as expected, to a reduction in secretion rate and to a decline in the cellular content of β-endorphin related peptides. This is consistent with in vivo studies in which ergocryptine has been shown to decrease the concentration of POMC mRNA [16] and the level of intracellular β-endorphin immunoreactivity [17] in the pars intermedia. In the presence of the dopaminergic agents, however, the reduced rates of POMC synthesis are accompanied by reduced rates of secretion and the β-endorphin-related peptides remaining in the cells may be more exposed to enzyme modulation, resulting in increased proteolysis and acetylation. Under these conditions it would be anticipated that the pattern of peptides produced in the dopamine-treated cells should be similar to that which exists in the pars intermedia in situ. This was in fact observed.

These studies demonstrate that dopaminergic agents influence the post-translational processing of β-endorphin-related peptides in the pars intermedia, in addition to affecting the level of secretion. The observation that the processing of β-endorphin 1–31 in the neuro-intermediate lobe of the pituitary responds to specific stimuli is consistent with the possibility that this peptide may fulfill a functional role in the periphery. Certainly the activity of pituitary β-endorphin is susceptible to modulation at the post-translational level.

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