The identification and characterization of α-*N*-acetylated β-endorphin in the human pituitary gland

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By using a radioimmunoassay specific for α-*N*-acetyl β-endorphin and its C-terminally shortened forms, we have established the presence of immunoreactive α-*N*-acetyl endorphin (irNacEP) in extracts of five post-mortem human pituitary glands (2.27 ± 0.64 ng/gland). This immunoreactivity has been further characterized by subjecting these extracts to reverse-phase high-performance liquid chromatography (RP-HPLC). In all cases the major peaks of irNacEP co-migrated with synthetic human standard α-*N*-acetyl α-endorphin (NacαEP), α-*N*-acetyl γ-endorphin (NacγEP) and NacβEP. These studies thus represent the initial demonstration that α-*N*-acetylation of β-endorphin and its shorter molecular forms occurs in the human pituitary gland.

Acetylation *β-Endorphin* *Human pituitary* *Reverse-phase HPLC* *Radioimmunoassay*

1. INTRODUCTION

β-Endorphin is a 31 amino acid opioid peptide derived from the 31-kDa proopiomelanocortin (POMC) precursor. It was first isolated from the porcine pituitary gland [1], and subsequently shown to be further processed to C-terminally shortened forms (βEP_{1-27}, βEP_{1-26}, βEP_{1-17} (γEP) and βEP_{1-16} (αEP) and/or -*N*-acetylated forms [2,3,4]. The pattern of post-translational processing to yield these various molecular forms differs between tissues and between species. For example, in the rat neuro-intermediate lobe (N-IL) the C-terminal derivatives of POMC are extensively acetylated, with the predominant species being the longer (1–31, 1–27 and 1–26) α-*N*-acetylated forms of β-endorphin; although the profile is qualitatively similar in the rat anterior pituitary (AP), the extent of acetylation is much less (< 5%) [5].

In sheep N-IL, β-endorphin is predominantly processed to the shorter (NacαEP, NacγEP and NacβEP_{1-27}) forms. Though the sheep AP has a profile similar to the N-IL, the extent of acetylation in sheep AP is much greater (25%) than in rat AP [5]. In bovine [6] and porcine [7] pituitaries α-*N*-acetylation is essentially confined to the N-IL, with only minimal acetylation of βEP occurring in the AP. For the monkey, there are conflicting data. Weber et al. [6] reported low (5%) levels of acetylated forms in the AP by radioimmunoassay; other workers, using similarly *N*-acetyl-specific antiserum, have found much higher levels of irNacEP (25%) in monkey anterior pituitary [8].

In human AP, βEP was first characterized as βEP_{1-31} with a small amount of βEP_{1-27} [9]. More recently, however, the C-terminally shortened forms (α- and γ-endorphin) have also been demonstrated [10]. In neither of these studies, however, was it possible to show the presence of any α-*N*-acetylated species. Here we have used specific α-*N*-acetyl endorphin radioimmunoassay and RP-HPLC, and by these means have established the presence of α-*N*-acetyl α-endorphin, α-*N*-acetyl γ-endorphin and α-*N*-acetyl β-endorphin in the human pituitary for the first time.

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2. MATERIALS AND METHODS

2.1. *Extraction*

Five human pituitaries were obtained at autopsy (10–48 h post-mortem) and extracted at 4°C in 2 ml of 0.1 M HCl by homogenizing with a Polytron (Brinkman Instruments, Westbury, NY; speed setting 2.5, 1×5 s burst). Homogenates were centrifuged (15000×*g*, 30 min at 4°C); supernatants were either diluted for radioimmunoassay (RIA) or 100 μl aliquots subjected to RP-HPLC.

2.2. *HPLC*

Extracts were chromatographed on a Nova Pak C-18 radial compression column (Waters Assoc.) as described [11]. The column was eluted with a linear gradient of 30–80% B for 30 min at 1 ml/min, where A is 11 mM trifluoroacetic acid (TFA), 3.6 mM acetic acid and B is 11 mM TFA containing 70% acetonitrile. The eluant was monitored at 214 nm. Fractions (0.5 ml) were evaporated and redissolved in the appropriate buffer prior to RIA.

2.3. *Radioimmunoassay*

The antiserum (R92) used for the radioimmunoassay has been described in [12]. It was raised against synthetic bovine α-*N*-acetyl βEP_{1-27}, and does not recognize αMSH, *N*-acetylated growth hormone fragments (provided by Professor J. Bornstein, Monash University), any of the enkephalins or any non-acetylated POMC-derived peptides; specifically, no displacement of tracer is seen with 1 μg of βEP_{1-31}, αEP or γEP. The antibody, however, cross-reacts 100% on a molar basis with α-*N*-acetyl αEP and longer acetylated forms.

3. RESULTS

Table 1 shows the levels of NacEP in extracts of 5 pituitaries. There appears to be no significant correlation with age or time after death. Fig.1 shows a typical profile of irNacEP in a human pituitary extract. The major peak of immunoreactivity elutes in the position of hNacβEP, with two smaller peaks eluting with hNacαEP and hNacγEP. Three smaller peaks (1–3), although not formally characterized, elute in the positions of the methionine sulphoxide forms of human NacαEP, NacγEP and NacβEP. This oxidation is possibly an artifact of the extraction procedure.

Table 1

Levels of immunoreactive NacEP in extracts of 5 pituitaries

	Age	Sex	PM delay (h)	irNacEP (ng/gland)
1	82	M	24	2.29
2	66	M	13	1.92
3	83	M	48	1.44
4	59	M	14	3.15
5	82	M	10	2.57

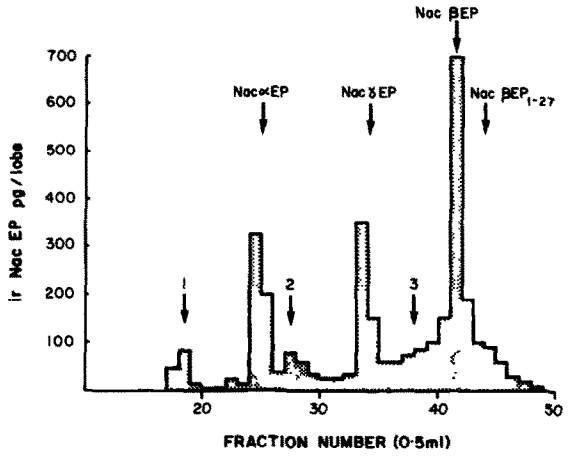

Fig.1. Profile of *N*-acetyl endorphin immunoreactivity after RP-HPLC of acid extracts of human pituitary tissue. Fractions were collected at 0.5 min intervals; the elution time of synthetic (h) standards were determined by UV absorbance (214 nm) in separate runs.

4. DISCUSSION

The human pituitary gland is unique in that it lacks a discrete pars intermedia, the classical site of α-*N*-acetylation of β-endorphin in lower mammals. Here we have demonstrated the presence of irNacEP in the human pituitary gland, though the levels represent ≪1% of the total pituitary ir-endorphin. The RP-HPLC profile of irNacEP appears to mirror that of non-acetylated βEP already characterised in the anterior pituitary [9,10], with NacβEP being the major species and two slightly lesser peaks of NacαEP and NacγEP. Unlike other species

studied, there is little βEP_{1-27} or NacβEP_{1-27}, and no βEP_{1-26} or NacβEP_{1-26}. This is presumably due to substitution of the histidyl residue at position 27 in other species by tyrosine, which is less susceptible to general endopeptidase activity and removal by exopeptidases.

In no species have physiological roles for pituitary βEP or its shorter/acetylated forms been established. It has been shown that α-*N*-acetylation of βEP and its C-terminally shortened fragments renders them opiate-receptor inactive [4]. However, there have been reported specific receptors recognising the C-terminus of βEP, its N-terminally shortened forms, and (by extrapolation) its α-*N*-acetylated forms [13]. Since the acetylation of the N-terminal tyrosyl residue protects the peptide against aminopeptidase degradation, *N*-acetylated βEP_{1-31} may thus represent a relatively stable ligand for such C-terminus specific receptors. An alternative role of *N*-acetylation, particularly for the shorter fragments (α- and γ-EP) may also be in preventing the formation of des-tyr forms, which have been shown, for example, to have specific pharmacological effects [14,15].

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