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COMMENTARY

MOLECULAR MECHANISMS OF β-ENDORPHIN BIOSYNTHESIS

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β-Endorphin: A member of the pro-opiomelanocortin peptide family

β-Endorphin is a member of the pro-opiomelanocortin (POMC†) family of peptides which also includes adrenocorticotropin ($ACTH_{1-39}$), α-melanocyte stimulating hormone (α-MSH) and β-lipotropin (β-LPH) (see Fig. 1). These peptides are synthesized primarily in the intermediate and anterior pituitary and brain [1–3] from a common precursor, POMC, by proteolytic cleavage at paired basic residues in a sequential manner [2]. POMC, a 31,000 molecular weight glycoprotein, is first cleaved between ACTH and β-LPH to yield a 21–23 kDa ACTH (N-POMC + $ACTH_{1-39}$) and β-LPH (Fig. 1). β-LPH is, in turn, cleaved to yield γ-LPH and β-$endorphin_{1-31}$, while the 21–23 kDa ACTH is cleaved to form a 16 kDa glycopeptide (N-POMC) and $ACTH_{1-39}$ (Fig. 1). In some tissues, $ACTH_{1-39}$ is further processed to α-MSH and the 16 kDa glycopeptide is processed to yield the joining peptide, γ-MSH and N-$POMC_{1-49}$. β-$Endorphin_{1-31}$ is the opioid active form of the peptide [4, 5]. However, in some cells, β-$endorphin_{1-31}$ undergoes α-N-acetylation and/or truncation to yield acetylated and non-acetylated forms of β-$endorphin_{1-27}$ and β-$endorphin_{1-26}$ (Fig. 2) which are opioid inactive [4]. In addition a dipeptide, Gly-Gln (Gly-Glu, in humans), is liberated from the cleavage at the Lys_{28}-Lys_{29} residues of β-$endorphin_{1-31}$ (see Fig. 1). In brain, small amounts of γ-endorphin (β-$endorphin_{1-17}$) and α-endorphin (β-$endorphin_{1-16}$) have been found. Although both α- and γ-endorphin retain opioid activity, and produce distinct behavioral effects thought to be mediated by a separate class of non-opioid receptors specific for these peptides [6, 7], they may represent degradation products. In this commentary, the regulation and enzymology of POMC processing will be discussed with specific emphasis on β-endorphin biosynthesis.

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† Abbreviations: POMC, pro-opiomelanocortin; ACTH, adrenocorticotropin; MSH, melanocyte stimulating hormone; LPH, lipotropin; PCE, pro-opiomelanocortin converting enzyme; CPH, carboxypeptidase H; OMAT, opiomelanotropin acetyltransferase; PHM, peptidylglycine α-hydroxylating monooxygenase; PAL, peptidyl-α-hydroxyglycine α-amidating lysate; and PAM, peptidylglycine α-amidating monooxygenase.

Enzymology of POMC and β-endorphin processing

POMC, like other pro-hormones and pro-neuropeptides, is biologically inactive, and it is processed at paired basic residues to yield various active peptides (Fig. 1). Several enzyme candidates have been described which can process POMC at paired basic residues. The first enzyme shown to cleave intact POMC correctly is proopiomelanocortin converting enzyme (PCE), purified from bovine pituitary intermediate lobe secretory granules [8, 9], the site of POMC processing [10]. PCE is a 70 kDa glycoprotein, and is characterized as a Ca^{2+}-activated, aspartic protease, with an acidic pH optimum [8, 9, 11]. PCE has been shown to cleave at the Lys-Arg and Arg-Lys paired basic residue sites of POMC, as indicated in Fig. 1. While PCE generates β-$endorphin_{1-31}$ from β-LPH, it does not cleave the Lys_{28}-Lys_{29} pair to yield β-$endorphin_{1-27}$ or β-$endorphin_{1-26}$. To date, no enzyme has been found that specifically cleaves this site to yield truncated forms of β-endorphin.

Recently, two other paired basic residue-specific enzymes, PC1 and PC2, have been reported. These enzymes were cloned from mouse pituitary and human insulinoma cells [12–15] using oligonucleotide probes designed from the cDNA sequence of KEX-2, the yeast paired basic residue-specific enzyme that processes pro-α-mating factor. By co-transfection of the cDNAs of PC1, PC2 and POMC into BSC 40 cells (a monkey kidney cell line), AtT-20 cells (a mouse pituitary tumor cell line), and chromaffin cells, these enzymes were shown to process POMC at various paired basic residue sites as indicated in Fig. 1 but they did not cleave β-$endorphin_{1-31}$ to yield shorter forms [16, 17]. Characterization of the cDNA encoding PC1 and PC2 indicates that they are Ca^{2+}-activated, subtilisin-like serine proteases, similar to KEX2 but lacking the transmembrane domain. PC1 and PC2 enzymes have been isolated from bovine chromaffin granules and anglerfish islet granules and identified by N-terminal sequencing [18, 19]. These studies revealed a size of 66 kDa for bovine chromaffin granule PC2 and two forms with a molecular weight of 57 and 65–67 kDa for anglerfish PC2 [18, 19].

PC2 has been expressed in *Xenopus* oocytes and the activity characterized using short fluorogenic peptide substrates [20]. The enzyme has a pH optimum of 5.5 and is poorly inhibited by the serine protease inhibitors phenylmethylsulfonyl fluoride and diisopropylfluorophosphate [20]. Recently, we

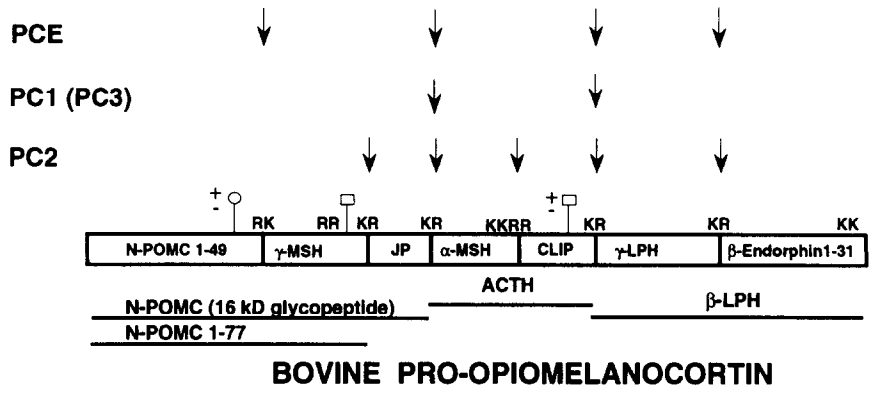

Fig. 1. Diagrammatic representation of bovine pro-opiomelanocortin. The cleavage sites denoted by single letter code and various processed products are indicated. The arrows indicate the sites cleaved by the three processing enzyme candidates pro-opiomelanocortin converting enzyme (PCE), PC2 and PC1 (see text). Abbreviations and symbols: POMC, pro-opiomelanocortin; LPH, lipotropin; MSH, melanocyte stimulating hormone; ACTH, adrenocorticotropin; JP, joining peptide; CLIP, corticotropin-like intermediate peptide; K, Lys; R, Arg, ○, O-linked glycosylation at Thr_{45}, and □, N-linked glycosylation sites. The KKRR residues represent amino acids 16–18 in $ACTH_{1-39}$ and the KK residues represent amino acids 28 and 29 in β-endorphin$_{1-31}$.

have reported a PC2-like enzyme activity in bovine intermediate lobe secretory granule membranes which cleaved POMC at paired basic residues to yield β-endorphin at pH 6.0. However, this activity cleaved $ACTH_{1-39}$ at the tetrabasic residues only at neutral pHs [21]. The PC2-like activity appears to exhibit differences in pH optimum for different substrates. In addition to the PC2-like activity, there is a tetrabasic residue-specific enzyme activity with an acidic pH optimum, in bovine intermediate lobe secretory granules that can cleave $ACTH_{1-39}$ to $ACTH_{1-17}$ [21]. $ACTH_{1-17}$ is subsequently converted to α-MSH (α,N-acetyl $ACTH_{1-13}$ NH_2) by removal of the basic residues 15–17, followed by C-terminal amidation and N-terminal acetylation. This acidic ACTH converting enzyme activity is a Ca^{2+}-activated serine protease, distinct from PC2 [21].

Clearly, there are several enzymes which can process POMC at paired and tetrabasic residues. Since POMC processing may be initiated at the trans-Golgi network [22], which is less acidic (pH 6.0 to 7.0), and continues in the secretory granules which have a more acidic internal pH (4 to 5.5) [10, 23, 24], multiple enzymes with different pH optima may have evolved for action at different intracellular sites to ensure the most efficient processing of POMC.

Endoproteolytic processing of POMC at paired basic residues liberates peptides with C-terminal extended basic amino acids since these enzymes cleave primarily on the carboxyl side of the Lys-Arg pairs [8, 20]. These basic residues are removed by a carboxypeptidase B-like enzyme, also known as carboxypeptidase E and carboxypeptidase H (CPH). CPH, found in secretory granules of various endocrine tissues and in brain, has been purified from pituitary secretory granules and cloned [25, 26]. This enzyme has a pH optimum of 5.5, is stimulated by Zn^{2+} and Co^{2+}, and is distinct from pancreatic carboxypeptidase B [for a review see Ref. 27]. In addition to the removal of the C-terminal basic residues, CPH also cleaves the His_{27} from β-endorphin$_{1-27}$ to yield β-endorphin$_{1-26}$ (Fig. 2) [28]. An aminopeptidase B-like enzyme that can remove specifically the N-terminal extended basic residudes from POMC

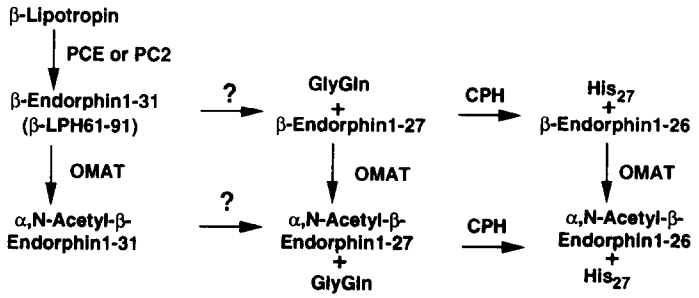

Fig. 2. Secondary processing of β-endorphin. Abbreviations: PCE pro-opiomelanocortin converting enzyme; CPH, carboxypeptidasde H; and OMAT, opiomelanotropin acetyltransferase.

peptides has been found in bovine intermediate lobe secretory granules and characterized. This enzyme has a pH optimum of 6.0 and is stimulated by Co^{2+} and Zn^{2+} [29].

The POMC peptides, β-endorphin and α-MSH, also undergo N-terminal modification in some tissues. They exist in the α,N-acetylated and non-acetylated forms [30, 31]. α,N-Acetylation greatly affects the bioactivity of these peptides. α-MSH, which is N-acetylated, has more potent melantropic activity than the des-acetyl form [32]. α,N-Acetylation of β-endorphin$_{1-31}$ elminates the opiate activity of this peptide [4]. An acetyltransferase activity which can acetylate both ACTH$_{1-14}$ and β-endorphin has been found in secretory granules of rat intermediate pituitary [33, 34]. This enzyme activity, also referred to as opiomelanotropin acetyltransferase (OMAT) [33, 34], has a pH optimum of 6.3 [34].

Some POMC-derived peptides, e.g. α-MSH, γ-MSH and joining peptide (see Fig. 1), also undergo C-terminal amidation. These peptides end in glycine at the C-terminus [35]. The glycine is converted to an amide by a two-step reaction:

$$\text{X-Gly} \xrightarrow{\text{E1}} \text{X-}\alpha\text{-hydroxyglycine} \xrightarrow{\text{E2}} \text{X-amide} + \text{glyoxylic acid}$$

where enzyme E1 is peptidylglycine α-hydroxylating monooxygenase (PHM) and enzyme E_2 is peptidyl-α-hydroxyglycine α-amidating lyase (PAL). It became evident that both these enzymes are synthesized from a common precursor known as peptidylglycine α-amidating monooxygenase (PAM) when the cDNA of this latter enzyme was cloned [36, 37]. The PHM catalytic domain is encoded in the N-terminal third of the molecule followed by an intragranular region. This is followed by the PAL catalytic domain, transmembrane domain and a cytoplasmic tail. PHM activity requires molecular oxygen, Cu^{2+} and ascorbic acid as cofactors and has a pH optimum of 5–8 depending on the substrate [36]. PAL activity has a pH optimum of 5 and is resistant to thiol reagents and urea but is inhibited by ethyleneglycolbis(aminoethylether)tetra-acetate (EGTA). PAM activity has been found in a number of endocrine and neuronal tissues such as pituitary and the hypothalamus [37], and appears to be the enzyme responsible for the amidation of POMC-derived peptides.

Tissue-specific processing of POMC and β-endorphin

POMC is processed differentially in the brain and the intermediate and anterior lobes of the pituitary. In the rat anterior pituitary, the major processed products are N-POMC$_{1-74}$, joining peptide, ACTH$_{1-39}$, and β-lipotropin (see Fig. 1) [2]. A small amount of β-endorphin, primarily in the opioid active form (β-endorphin$_{1-31}$), has also been found in the anterior pituitary [38]. In the intermediate pituitary, the major forms of POMC peptides are N-POMC$_{1-49}$, γ-MSH, joining peptide, α-MSH and β-endorphin [2]. All the six forms of β-endorphin peptides (Fig. 2) are present in the intermediate pituitary [38]. In contrast to anterior pituitary, β-endorphin$_{1-31}$ is a minor component (<100%), while the α, N-acetyl forms of β-endorphin$_{1-26}$ and β-endorphin$_{1-27}$ predominate [38]. This pattern of POMC processing in the two lobes of the pituitary of rats extends to other species as well [38]. In humans, which lack a pars intermedia, the major form of β-endorphin in the pituitary is the opioid active form, the acetylated forms being virtually absent. While some β-endorphin$_{1-27}$ is present, β-endorphin$_{1-26}$ and its acetyl derivative do not exist in the human pituitary, presumably because in this species the His$_{27}$ is replaced by Tyr which is less susceptible to removal by carboxypeptidase H (CPH) [28].

In the brain, the pattern of POMC processing is similar to the intermediate pituitary. The major peptides are N-POMC$_{1-49}$, α-MSH, joining peptide, des-acetyl α-MSH and β-endorphin [3]. The main concentration of β-endorphin in the brain is in the hypothalamus where the cell bodies are confined to the arcuate nucleus, median eminence and the ventromedial border of the third ventricle [38]. From the hypothalamus, the axons of the POMC cell bodies extend dorsally and laterally with the terminals ending in the amygdala, colliculae and hippocampus. Fibers and terminals have also been observed in the periaqueductal grey region [38]. The predominant form of β-endorphin in the rat hypothalamus is the opioid active form, β-endorphin$_{1-31}$. Similarly, β-endorphin$_{1-31}$ is the major form in the midbrain and the amygdala, although a significant amount of β-endorphin$_{1-26}$ is also present. These regions seem to have very little acetylated forms. In contrast, the hippocampus, colliculae and brain stem contain primarily α,N-acetyl β-endorphin$_{1-27}$ and α,N-acetyl β-endorphin$_{1-26}$; the α,N-acetyl β-endorphin$_{1-31}$, its bioactive form, β-endorphin$_{1-31}$, and the truncated forms are present to a lesser extent [38].

In the dorsal medulla, the site of the nucleus tractus solitarius POMC cell bodies, β-endorphin$_{1-31}$, β-endorphin$_{1-27}$ and the acetylated forms of both these peptides are present. Approximately 65% of the total β-endorphin is acetylated, 50% of which is represented by α,N-acetyl β-endorphin$_{1-27}$ [3, 39–41].

The pattern of distribution of β-endorphin forms in the different brain regions is rather intriguing. In the hypothalamus where the POMC neuronal cell bodies and the short axonal projections are localized, the longer opioid active forms of β-endorphin are predominant, yet in the brain areas where the terminals of the more distal axonal projections of the same neurons are found, β-endorphin exists primarily in the non-opioid, truncated and acetylated forms. It is possible that the existence of shorter, acetylated forms in the distal projections may be time dependent, reflecting the duration of their exposure to the specific processing enzymes, since continuous processing occurs within the secretory granules during axonal transport and storage [42]. However, the highly specific nature of the products formed in the different brain regions, and the multiplicity of enzymes necessary for their formation suggest that distance from the site of biosynthesis (the cell body) is unlikely to account for the organized distribution of the various forms of β-endorphin. Alternatively, there is a heterogenous population of POMC neurons in the hypothalamus, and only the

ones that make the shorter and acetylated forms of β-endorphin project to the distal areas.

Regulation of β-endorphin biosynthesis

β-Endorphin biosynthesis appears to be regulated at the transcriptional [43, 44] and post-translational level [45]. Treatment of rats with haloperidol, a dopamine antagonist which stimulates secretion of POMC peptides from the intermediate pituitary, resulted in up-regulation, whereas bromocryptine, which inhibits secretion, resulted in down-regulation of POMC mRNA and β-endorphin biosynthesis [43–48]. The pattern of processing of β-endorphin was also modulated. Haloperidol treatment increased the acetylated derivatives: α,N-acetyl β-endorphin$_{1-27}$ and α,N-acetyl β-endorphin$_{1-31}$, and to a much lesser extent α,N-acetyl β-endorphin$_{1-26}$, with no change in the level of the NH_2 peptides [46, 47]. However, bromocryptine treatment did not alter the pattern of β-endorphin processing significantly [46, 48]. This is perhaps not surprising since *in vivo*, biosynthesis and secretion of POMC peptides in the pars intermediate are under inhibitory control by dopamine [45, 49, 50]. These results demonstrate that dopaminergic agents can regulate POMC biosynthesis and post-translational modification of β-endorphin in the intermediate lobe. Stress, and other physiological stimuli which affect dopamine secretion, have been shown to alter biosynthesis and processing of β-endorphin in the pars intermediate. For example, chronic stress followed by rest resulted in an increase in β-endorphin$_{1-31}$ and its acetylated form, while α,N-acetyl β-endorphin$_{1-27}$ decreased relative to control [51]. Black and white background adaptation of the frog *Xenopus laevis* also resulted in differences in β-endorphin processing in pars intermedia. The predominant form in white adapted frogs was α,N-acetyl β-endorphin$_{1-8}$, whereas in black adapted frogs there were approximately equal amounts of α,N-acetyl β-endorphin$_{1-8}$ and the longer acetylated forms [52].

β-Endorphin levels and processing in the pars intermedia are also regulated by T-cell-specific factors secreted in animals undergoing transplantation immunity [53]. Allografted rats showed a 3-fold increase in the level of β-endorphin immunoreactivity as compared to normal and isografted rats. While in normal rats and rats with an isogenic skin graft the α,N-acetyl forms of β-endorphin$_{1-27}$ and β-endorphin$_{1-26}$ were the predominant peptides, rats after injection of a second allograft showed the presence of primarily β-endorphin$_{1-31}$ and α,N-acetyl β-endorphin$_{1-31}$ in the pars intermedia. Injection of the T-cell factor, interleukin 2, to athymic rats gave a pattern of β-endorphin peptides mimicking that of the allografted rats.

Concluding remarks

As discussed in this review, much is now known about the mechanism of processing and the enzymes involved in the biosynthesis of various forms of β-endorphin and other POMC-derived peptides. Moreover, it is also well documented that processing of POMC and β-endorphin is tissue-specific, and that the "steady state" pattern of β-endorphin processed products in a given tissue can be modulated under different physiological conditions. However, two important issues remain only partially resolved, although much insight has been gained recently. These are: (1) what are the mechanisms regulating the differential processing of POMC and β-endorphin? and (2) what is the functional significance of the various products generated from the differential secondary processing of β-endorphin?

The identification of the paired basic residue-specific processing enzymes has facilitated studies addressing the issue of the mechanisms regulating the differential processing of POMC. Several mechanisms have been proposed and in some cases supported by experimental data.

(1) Tissue-specific "O"-linked glycosylation of POMC at a residue close to the cleavage site can influence the processing at that site. This proposal was based on the findings by Bennett's group that N-POMC present in anterior pituitary was O-glycosylated at threonine$_{45}$ (see Fig. 1), whereas the cleaved product N-POMC$_{1-49}$ isolated from intermediate lobe was not O-glycosylated [54]. *In vitro* experiments, incubating PCE with bovine N-POMC$_{1-77}$ which was either O-glycosylated at the Thr$_{45}$ or lacking O-glycosylation at this site provided evidence for this mechanism [55]. Non-O-glycosylated N-POMC was cleaved by PCE to yield N-POMC$_{1-49}$ and γ-MSH, but fully O-glycosylated N-POMC$_{1-77}$ was not. The O-linked glycosylation near the Arg$_{49}$-Lys$_{50}$ appears to have prevented cleavage of this site, probably by causing stearic hindrance to the enzyme. Thus, the differential O-linked glycosylation of the Thr$_{45}$ of POMC in the anterior versus intermediate lobe provides a mechanism for the differential processing of POMC at the Arg$_{49}$-Lys$_{50}$ site in these two lobes.

(2) The microenvironment (e.g. Ca^{2+} concentration, pH, and the enzyme to substrate ratio) within the intragranular compartment where processing occurs may vary in different tissues and thus influence the degree of processing of POMC by the enzymes. Studies have shown that the extent of processing β-LPH to β-endorphin$_{1-31}$ by PCE *in vitro* is influenced by the enzyme/substrate [9] and the Ca^{2+} concentration [11]. Possible differences in the intragranular ratio of processing enzyme to substrate concentration and/or Ca^{2+} concentration in the intermediate and anterior lobe secretory granules may account for the differential amounts of β-endorphin versus β-LPH in these two tissues. Cation channels which are regulated by Ca^{2+} and can act as cation transporters to alter intragranular Ca^{2+} have been found in pituitary secretory granules [56]. Furthermore, although in a different opiate system, it has been shown that electrical stimulation, which can alter intragranular Ca^{2+}, enhanced the processing of larger forms of met-enkephalin in guinea pig myenteric plexus [57]. All these factors support the proposal that differences in the microenvironment may regulate the extent of processing of peptides from larger forms.

(3) Expression of different enzymes for various post-translational modification events in a tissue-specific manner can provide another mechanism for tissue-specific differential processing. It is known that in the intermediate lobe, β-LPH is completely

processed to β-endorphin, whereas in the anterior lobe, β-LPH is the major product. This pattern of processing correlates well with the presence of much larger amounts of PC2 mRNA and presumably enzyme, which cleaves β-LPH to β-endorphin, in the intermediate than in the anterior lobe (see Fig. 1), whereas PC1 (PC3) mRNA, encoding an enzyme which does not generate β-endorphin from POMC, is about equally distributed in the two lobes [15, 58]. Moreover, PC2 mRNA has also been shown to be coordinately regulated with POMC mRNA by a dopamine agonist and an antagonist in the intermediate lobe [58]. Another enzyme that is differentially distributed is the acidic ACTH converting enzyme which generates α-MSH. There appears to be greater amounts of this enzyme in the intermediate versus the anterior lobe, which synthesizes little or no α-MSH [21]. Analysis of the activity of OMAT in the intermediate and anterior pituitary has also indicated selective expression of this enzyme primarily in the intermediate lobe [34]. Thus, the tissue-specific expression of OMAT in the intermediate lobe is consistent with the presence of acetylated forms of β-endorphin, only in this lobe, and not in the anterior lobe [38]. Processing enzyme studies suggest that the cleavage of the Lys_{28}-Lys_{29} pair in β-endorphin$_{1-31}$ to yield β-endorphin$_{1-27}$ is carried out by a distinct yet unidentified enzyme. Once an enzyme specific for this cleavage is identified, it will be possible to determine if it is present specifically in tissues which yield the truncated forms of β-endorphin$_{1-31}$.

Mechanisms for tissue-specific processing of POMC and β-endorphin proposed above are derived from studies on the anterior and intermediate lobe. It is likely that such mechanisms extend to the nervous system as well. One of the remaining tasks relating to β-endorphin biosynthesis is the identification of the enzyme responsible for the cleavage of Lys_{28}-Lys_{29} of β-endorphin$_{1-31}$ to yield β-endorphin$_{1-27}$. Purification cloning and production of specific antibodies to the processing enzymes in the not too distant future will facilitate quantitative studies of the enzymes, and will lead to a better understanding of the enzymatic mechanisms underlying differential processing and modulation of β-endorphin biosynthesis at the post-translational level.

The second issue concerns the functional significance of the differential secondary processing of β-endorphin$_{1-31}$. Tissue-specific processing of POMC and the secondary processing of β-endorphin$_{1-31}$ allow the versatility of using a single gene to generate multiple products with different biological activities. Some of these products may potentiate the activity of another, e.g. β-endorphin$_{1-31}$ was found to enhance melantropic activity [59], whereas β-endorphin$_{1-27}$ antagonizes the opiate activity of β-endorphin$_{1-31}$ with a potency five times greater than naloxone [60]. β-Endorphin$_{1-26}$, on the other hand, was inefficient in reversing analgesia [60]. Processing of β-endorphin$_{1-31}$ to β-endorphin$_{1-27}$ also yields a dipeptide, Gly_{30}-Gln_{31}, which has been found in secretory granules of the pars intermedia and brain stem where truncated forms of β-endorphin predominate [38]. This dipeptide was shown to inhibit, in a dose-dependent manner, the firing of neurons located within the nucleus reticularis gigantocellaris and nucleus reticularis pontis caudalis of the brain stem reticular formation [61]. More recently, various forms of β-endorphin were tested for their ability to modify central cardioregulation in the rat caudal medulla [62], since this site is richly innervated by two distinctive β-endorphin projections, one from cell bodies in the hypothalamus which produces primarily non-acetylated peptides and the other from the nucleus tractus solitarus which contains both desacetyl and acetylated forms [39–41]. β-Endorphin peptides were injected intracisternally and the mean arterial pressure was measured. β-Endorphin$_{1-27}$ was found to be 10-fold more potent than β-endorphin$_{1-31}$ in lowering mean arterial pressure. However, β-endorphin$_{1-26}$ and the α,N-acetyl forms of β-endorphin$_{1-31}$, β-endorphin$_{1-27}$ and β-endorphin$_{1-26}$, were inactive. The hypotensive effect of β-endorphin$_{1-27}$ and β-endorphin$_{1-31}$ was blocked by naloxone, suggesting that both these peptides act through opioid receptors.

Studies continue to elucidate the biological function of truncated and α-N-acetylated forms of β-endorphin. At present it would appear that while β-endorphin$_{1-27}$ has biological activity, β-endorphin$_{1-26}$ is inactive in the functions tested. Removal of $histidine_{27}$ from β-endorphin$_{1-27}$ may be an inactivating step. The function of α,N-acetylated forms of β-endorphin is also poorly understood. It would be expected that these forms are not inactive; rather their biological function(s) is awaiting discovery.

In conclusion, the processing of POMC to yield β-endorphin$_{1-31}$, and the secondary processing of β-endorphin$_{1-31}$ (see Fig. 2) yield six endorphin peptides, the formation of which is region-specific and can be modified by physiological stimuli. The forms released by a given cell are probably designed for the action they have to perform in response to a specific stimulus. Understanding the regulation of tissue- and stimulus-specific processing of β-endorphin$_{1-31}$, the biological role of the various truncated and α,N-acetylated forms of β-endorphin, and the interplay between the opioid active and antagonist forms of β-endorphin remains a major challenge for the future.

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REFERENCES

1. Crine P, Gossard F, Seidah NG, Blanchette L, Lis M and Chrétien M, Concomitant synthesis of β-endorphin and α-melanotropin from two forms of pro-opiomelanocortin in the rat pars intermedia. *Proc Natl Acad Sci USA* **76**: 5085–5089, 1979.
2. Eipper BA and Mains RE, Structure and biosynthesis of pro-adrenocorticotropin/endorphin and related peptides. *Endocr Rev* **1**: 1–27, 1980.
3. Emeson RB and Eipper BA, Characterization of pro-ACTH/endorphin-derived peptides in rat hypothalamus. *J Neurosci* **6**: 837–849, 1986.
4. Deakin JF, Dostrovsky JO and Smyth DG, Influence of N-terminal acetylation and C-terminal proteolysis on the analgesic activity of β-endorphin. *Biochem J* **189**: 501–506, 1980.
5. Loh HH, Tseng LF, Wei E and Li CH, β-Endorphin is

a potent analgesic agent. *Proc Natl Acad Sci USA* **73**: 2895–2898, 1976.
6. Burbach JPH, Loeber JG, Verhoef J, Wiegant VM, de Kloet ER and de Wied D, Selective conversion of β-endorphin into peptides related to γ- and α-endorphin. *Nature* **283**: 96–97, 1980.
7. De Wied D and Jolles J, Neuropeptides derived from pro-opiocortin: Behavioral, physiological and neurochemical effects. *Physiol Rev* **62**: 976–1059, 1982.
8. Loh YP, Parish DC and Tuteja R, Purification and characterization of a paired basic residue-specific pro-opiomelanocortin converting enzyme from bovine pituitary intermediate lobe secretory vesicle. *J Biol Chem* **260**: 7194–7205, 1985.
9. Loh YP, Kinetic studies on the processing of human β-lipotropin by bovine pituitary intermediate lobe pro-opiomelanocortin converting enzyme. *J Biol Chem* **261**: 11949–11955, 1986.
10. Tooze J, Hollinshead M, Frank R and Burke B, An antibody specific for an endoproteolytic cleavage site provides evidence that pro-opiomelanocortin is packaged into secretory granules in AtT20 cells before its cleavage. *J Cell Biol* **105**: 155–162, 1987.
11. Birch NP, Bennett HPJ, Estivariz FE and Loh YP, Effect of calcium ions on the processing of pro-opiomelanocortin by bovine intermediate lobe pro-opiomelanocortin-converting enzyme. *Eur J Biochem* **201**: 85–89, 1991.
12. Smeekens SP and Steiner DF, Identification of a human insulinoma cDNA encoding a novel mammalian protein structurally related to the yeast dibasic processing protease Kex2. *J Biol Chem* **265**: 2997–3000, 1990.
13. Seidah NG, Gaspar L, Mion P, Marcinkiewicz M, Mbikay M and Chrétien M, cDNA sequence of two distinct pituitary proteins homologous to Kex2 and furin gene products: Tissue-specific mRNAs encodeing candidates for pro-hormone processing proteinases. *DNA Cell Biol* **9**: 415–424, 1990.
14. Smeekens SP, Avruch AS, LaMendola J, Chan SJ and Steiner DF, Identification of cDNA encoding a second putative prohormone convertase related to PC2 at AtT20 cells and islets of Langerhans. *Proc Natl Acad Sci USA* **88**: 340–344, 1991.
15. Seidah NG, Marcinkiewicz S, Benjannet S, Gaspar L, Beaubien G, Mattei MG, Lazure C, Mbikay M and Chrétien M, Cloning and primary sequence of a mouse candidate prohormone convertase PC1 homologous to PC2 and Kex2: Distinct chromosomal localization and messenger RNA distribution in brain and pituitary compared to PC2. *Mol Endocrinol* **5**: 111–122, 1991.
16. Thomas L, Leduc R, Thorne BA, Smeekens SP, Steiner DF and Thomas G, Kex2-like endoprotease PC2 and PC3 accurately cleave a model prohormone in mammalian cells: Evidence for a common core of neuroendocrine processing enzymes. *Proc Natl Acad Sci USA* **88**: 5297–5301, 1991.
17. Benjannet S, Rondeau N, Day R, Chréitien M and Seidah NG, PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. *Proc Natl Acad Sci USA* **88**: 3564–3568, 1991.
18. Christie DL, Batchelor DC and Palmer DJ, Identification of kex2-related proteases in chromaffin granules by partial amino acid sequence analysis. *J Biol Chem* **266**: 15679–15683, 1991.
19. Mackin RB, Noe BD and Spiess J, Identification of a somatostatin-14-generating propeptide converting enzyme as a member of the kex2/furin/PC family. *Endocrinology* **129**: 2263–2265, 1991.
20. Shennan KIJ, Smeekens SP, Steiner DF and Docherty K, Characterization of PC2, a mammalian Kex2 homologue, following expression of the cDNA in microinjected *Xenopus oocytes*. *FEBS Lett* **284**: 277–281, 1991.
21. Estivariz FE, Friedman TC, Chikuma T and Loh YP, Processing of adrenocorticotrophin by two proteases in bovine intermediate lobe secretory vesicle membranes: A distinct acidic tetrabasic residue-specific calcium-activated serine protease and a PC2-like enzyme. *J Biol Chem* **267**: 7456–7463, 1992.
22. Schnabel E, Mains RE and Farquhar MG, Proteolytic processing of pro-ACTH/endorphin begins in the Golgi complex. *Mol Endocrinol* **3**: 1223–1235, 1989.
23. Orci L, Ravazzola M, Amherdt M, Madsen O, Perrahat A, Vassali JD and Anderson RGW, Conversion of pro-insulin to insulin occurs coordinately with acidification of maturing secretory vesicles. *J Cell Biol* **103**: 2273–2281, 1986.
24. Loh YP, Tam WWH and Russell JT, Measurement of ΔpH and membrane potential in secretory vessicles isolated from bovine pituitary intermediate lobe. *J Biol Chem* **259**: 8238–8245, 1984.
25. Hook VYH and Loh YP, Carboxypeptidase B-like converting enzyme activity in secretory granules of rat pituitary. *Proc Natl Acad Sci USA* **81**: 2776–2780, 1984.
26. Fricker LD, Evans CJ, Esch FS and Herbert E, Cloning and sequence analysis of cDNA for bovine carboxypeptidase E. *Nature* **323**: 461–464, 1986.
27. Fricker LD, Peptide processing exopeptidases: Amino- and carboxypeptidases involved with peptide biosynthesis. In: *Peptide Biosynthesis and Processing* (Ed. Fricker LD), pp. 199–249. CRC Press, Boca Raton, 1991.
28. Smyth DG, Maruthainar K, Darby NJ and Fricker LD, Catalysis of slow C-terminal processing reactions by carboxypeptidase H. *J. Neurochem* **53**: 489–493, 1989.
29. Gainer H, Russell JT and Loh YP, An aminopeptidase activity in bovine pituitary secretory vesicles that cleaves the N-terminal arginine from β-lipotropin$_{60-65}$. *FEBS Lett* **175**: 135–139, 1984.
30. Smyth DG, Massey DE, Zakarian S and Finnie MDA, Endorphins are stored in biologically active and inactive forms: Isolation of α-*N*-acetyl peptides. *Nature* **279**: 252–254, 1979.
31. Rudman D, Hollins BM, Kutner MH, Moffitt SD and Lynn MJ, Three types of α-melanocyte-stimulating hormone: Bioactivities and half-lives. *Am J Physiol* **245**: E47–E54, 1983.
32. Guttmann St and Boissonnas RA, Influence of the structure of the *N*-terminal extremity of α-MSH on the melanophore stimulating activity of this hormone. *Experientia* **17**: 265–267, 1961.
33. Glembotski CC, Characterization of the peptide acetyl transferase activity in bovine and rat intermediate pituitaries responsible for the acetylation of β-endorphin and α-melanotropin. *J Biol Chem* **257**: 10501–10509, 1982.
34. Chappell MC, Loh YP and O'Donohue T, Evidence for an opiomelanotropin acetyltransferase in the rat pituitary neurointermediate lobe. *Peptides* **3**: 405–410, 1982.
35. Nakanishi S, Inoue A, Kita T, Nakamura M, Chang ACY, Cohen SN and Numa S, Nucleotide sequence of cloned cDNA for bovine corticotropin-β-lipotropin precursor. *Nature* **278**: 423–426, 1979.
36. Eipper BA, Perkins SN, Husten EJ, Johnson RC, Keutmann JH and Mains RE, Peptidyl-α-hydroxyglycine α-amidating lyase. *J Biol Chem* **266**: 7827–7833, 1991.
37. Eipper BA and Mains RE, Peptide α-amidation. *Annu Rev Physiol* **50**: 333–334, 1988.
38. Zakarian S and Smyth DG, Distribution of β-endorphin-related peptides in rat pituitary and brain. *Biochem J* **202**: 561–571, 1982.
39. Dennis M, Seidah NG and Chrétien M, Regional het-

erogeneity in the processing of pro-opiomelanocortin in rat brain. *Life Sci* **33**(Suppl 1): 49–52, 1983.
40. Dores RM, Jain M and Akil H, Characterization of the forms of β-endorphin and α-MSH in the caudal medulla of the rat and guinea pig. *Brain Res* **377**: 251–260, 1986.
41. Zakarian S and Smyth DG, β-Endorphin is processed differently in specific regions of rat pituitary and brain. *Nature* **296**: 250–252, 1982.
42. Gainer H, Sarne Y and Brownstein MJ, Biosynthesis and conversion of a putative precursor during axonal transport. *Science* **195**: 1354–1356, 1977.
43. Chen CLC, Dionne FT and Roberts JL, Regulation of the pro-opiomelanocortin mRNA levels in rat pituitary by dopaminergic compounds. *Proc Natl Acad Sci USA* **80**: 2211–2215, 1983.
44. Hollt V, Haarmann I, Seizinger BR and Herz A, Chronic haloperidol treatment increases the level of *in vitro* translatable messenger ribonucleic acid coding for the β-endorphin/adrenocorticotropin precursor pro-opiomelanocortin in the pars intermediate of the rat pituitary. *Endocrinology* **110**: 1885–1891, 1982.
45. Hollt V and Bergman M, Effects of acute and chronic haloperidol treatment on the concentrations of immunoreactive β-endorphin in plasma, pituitary and brain of rats. *Neuropharmacology* **21**: 147–154, 1982.
46. Millington WR, O'Donohue TL and Mueller GP, Dopaminergic agents selectively alter the post-translational processing of β-endorphin in the intermediate pituitary of the rat. *J Pharmacol Exp Ther* **243**: 160–170, 1987.
47. Ham J and Smyth DG, β-Endorphin processing in pituitary and brain is sensitive to haloperidol stimulation. *Neuropeptides* **5**: 497–500, 1985.
48. Smith AI, Wallace CA, Clarke IJ and Funder JW, Dopaminergic agents differentially regulate both processing and content of α-N-acetylated endorphin and α-MSH in the ovine pituitary intermediate lobe. *Neuroendocrinology* **49**: 545–550, 1989.
49. Tilders FJH and Smelik PG, Direct neural control of MSH secretion in mammals: The involvement of dopaminergic tubero-hypophysial neurones. *Front Horm Res* **4**: 80–93, 1977.
50. Farah JM, Malcolm DS and Mueller GP, Dopaminergic inhibition of pituitary β-endorphin-like immunoreactivity secretion in the rat. *Endocrinology* **110**: 657–659, 1982.
51. Akil H, Shiomi H and Matthews J, Induction of the intermediate pituitary by stress: Synthesis and release of a nonopioid form of β-endorphin. *Science* **227**: 424–426, 1984.
52. Maruthainar K, Loh YP and Smyth DG, The processing of β-endorphin and melantropin in *Xenopus* par intermedia is influenced by background adaptation. *J Endocrinol*, in press.
53. Zakarian S, Eleazar MS and Silvers WK, Regulation of pro-opiomelanocortin biosynthesis and processing by transplantation immunity. *Nature* **339**: 553–556, 1989.
54. Seger MA and Bennett HPJ, Structure and bioactivity of the amino-terminal fragment of pro-opiomelanocortin. *J Steroid Biochem* **25**: 703–710, 1986.
55. Birch NP, Estivariz FE, Bennett HPJ and Loh YP, Differential glycosylation of N-POMC$^{1-77}$ regulates the production of γ_3-MSH by purified pro-opiomelanocortin converting enzyme. A possible mechanism for tissue-specific processing. *FEBS Lett* **290**: 191–194, 1991.
56. Lemos JR, Ocorr KA and Nordmann JJ, Possible role for ionic channels in neurosecretory granules of the rat neurohypophysis. In: *Secretion and its Control* (Eds. Oxford GS and Armstrong CM), pp. 333–347. Rockefeller University Press, New York, 1989.
57. Glass J, Clouet D and Gintzler AR, Short-term nerve stimulation increases enkephalin production and content in the guinea pig myenteric plexus. *Brain Res* **372**: 180–184, 1986.
58. Birch NP, Tracer HL, Hakes DJ and Loh YP, Coordinate regulation of mRNA levels of pro-opiomelanocortin and the candidate processing enzymes PC2 and PC3, but not furin, in rat pituitary intermediate lobe. *Biochem Biophys Res Commun* **179**: 1311–1316, 1991.
59. Carter RJ, Shuster SAM and Morley JS, Melanotropin potentiating factor is the C-terminal tetrapeptide of human β-lipotropin. *Nature* **279**: 74–75, 1979.
60. Nicolas P and Li CH, β-Endorphin-(1–27) is a naturally occurring antagonist to etorphine-induced analgesia. *Proc Natl Acad Sci USA* **82**: 3178–3181, 1985.
61. Parish DC, Smyth DG, Normanton JR and Wolstencroft JH, Glycyl glutamine, an inhibitory neuropeptide derived from β-endorphin. *Nature* **306**: 267–270, 1983.
62. Hirsch MD and Millington WR, Endoproteolytic conversion of β-endorphin-1-31 to β-endorphin-1-27 potentiates its central cardioregulatory activity. *Brain Res* **550**: 61–68, 1991.