[Frontiers in Bioscience 12, 3545-3553, May 1, 2007]

Discovery of new peptides from old prohormones: insights for energy balance and beyond

Lawrence R. Mulcahy[1,2], Eduardo A. Nillni[1]

[1] Division of Endocrinology, Department of Medicine Brown Medical School/Rhode Island Hospital, and Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI 02903. [2] Department of Microbiology and Molecular Genetics, Harvard Medical School, Microbiology-Armenise, Boston, MA 02115

TABLE OF CONTENTS

1. ABSTRACT

A complex network of peptide hormones secreted from the gut, adipose tissue, the brain, and other tissues regulate energy balance. Intensive investigation has uncovered the role of many of the major players in energy balance. However, many of these peptide hormones are derived from precursor proteins whose *in vivo* processing have not been fully studied. In this review we highlight the importance of fully understanding the processing of prohormones and highlight a particular case of why this is important with the recent discovery of the peptide obestatin.

2. INTRODUCTION

Hormonal and neuropeptide signaling play a major role in maintaining physiological homeostasis. After Steiner and colleagues discovered that insulin is synthesized as part of a larger precursor protein (1), or prohomone, it was realized that the majority of hormones and neuropeptides are synthesized first as prohormones and proneuropeptides which are endoproteolyzed and enzymatically modified to yield mature and bioactive peptides.

While proinsulin is known to have only one functional hormone derived from it, there are a number of prohormones that produce several peptides with known or suspected bioactivity. In this review we will highlight three such prohormones that play a prominent role in energy balance and regulation of obesity. What we aim to describe is the importance of fully studying the processing of prohormones and investigating the functionality of all associated peptides.

3. GENERAL INFORMATION ON PROHORMONE PROCESSING

Prohormone convertases (PC) are the enzymes that endoproteolyze prohormones into smaller peptides. These enzymes are similar to the yeast kexin and bacterial subtilisin and in fact were first identified by using the yeast kexin sequence (2). Several members of the PC family were subsequently identified and characterized and two convertases in particular, PC1/3 and PC2 were implicated in prohormone processing, a topic which has been previously been reported on in several excellent reviews (3-6). After endoproteolysis, the released peptides undergo

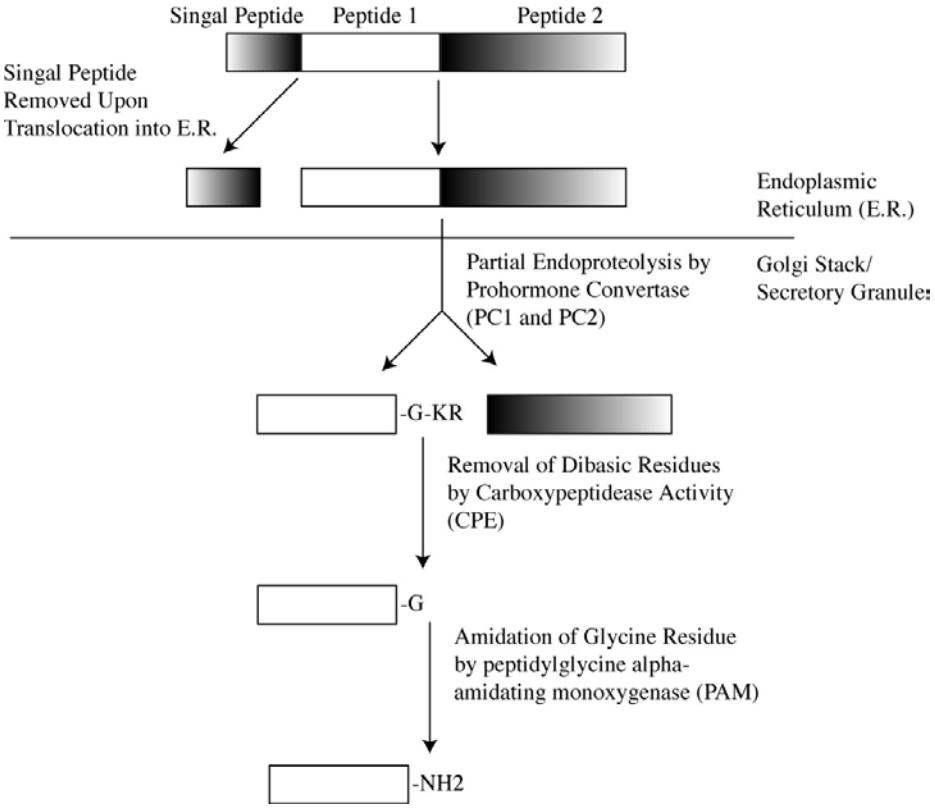

Figure 1. General depiction of precursor molecule processing. The enzymes primarily implicated in processing of the neuropeptides under consideration are in parantheses.

further enzymatic modification important for peptide function. PCs cleave on the carboxy-terminal side of dibasic residues leaving peptides with a pair of basic residues (generally Lys-Arg or Arg-Arg) extending from their carboxy-terminus. These basic residues are removed by a class of enzymes known as carboxypeptidases (CP). Two CP enzymes have been implicated in removal of dibasic residues from neuroendocrine peptides, CPD and CPE (7-11). Many neuropeptides also have a conserved glycine reside at their carboxy -terminus immediately preceding the dibasic sites of cleavage. This glycine is amidated by an enzyme known as peptidylglycine alpha-amidating monoxygenase (PAM), an important step for both function and stability of neuropeptides (12-16). A simplified scheme of this entire process is shown in Figure 1. These processes vary depending on the cohort of processing enzymes expressed in each specific tissue type where a given prohormone is expressed.

4. PROOPIOMELANOCORTIN

Study of andrenocorticotrophin (ACTH) led to the discovery that ACTH is derived from the precursor proopiomelanocortin (POMC) (17) and ultimately the cloning of the entire precursor gene (18). Further study of this important prohormone has revealed the generalized processing scheme for POMC (Figure 2). Peptides derived from POMC are involved in energy balance (alpha-MSH), adrenal gland development and maintenace (N-POMC), steroidogenesis (ACTH), and pigmentation (ACTH, alpha-MSH, gamma-LPH).

Biogenesis of the numerous POMC-derived peptides varies depending on the tissue in which POMC is expressed. There are several main sites of POMC biosynthesis in the body: the pituitary, the hypothalamus, and the placenta. Of main concern here is POMC produced in the pituitary and hypothalamus.

As shown in Figure 2 POMC contains eight consensus sights for endoproteolysis by convertases. In the anterior pituitary only four of these sites are processed thus primarily producing ACTH in addition to N-POMC, joining peptide (JP), beta-LPH, and small amounts of gamma-LPH and beta-endorphin due to inefficient cleavage of beta-LPH (19-21). The anterior pituitary primarily expresses PC1 and no PC2 which explains why POMC is not maximally processed here (22-24). The intermediate lobe of the pituitary expresses PC2 in addition to PC1 and thus the primary product here is alpha-MSH in addition to the production of beta-MSH from gamma-LPH, beta-end 1-27 from beta-endorphin, CLIP and gamma-MSH. Most of these smaller peptides are released from precursors due to PC2 processing. In the well documented case of the PC2 mouse knockout animal, alpha-MSH and beta-endorphin are not detectable and there is a buildup in ACTH and POMC precursor in the pituitary (25, 26).

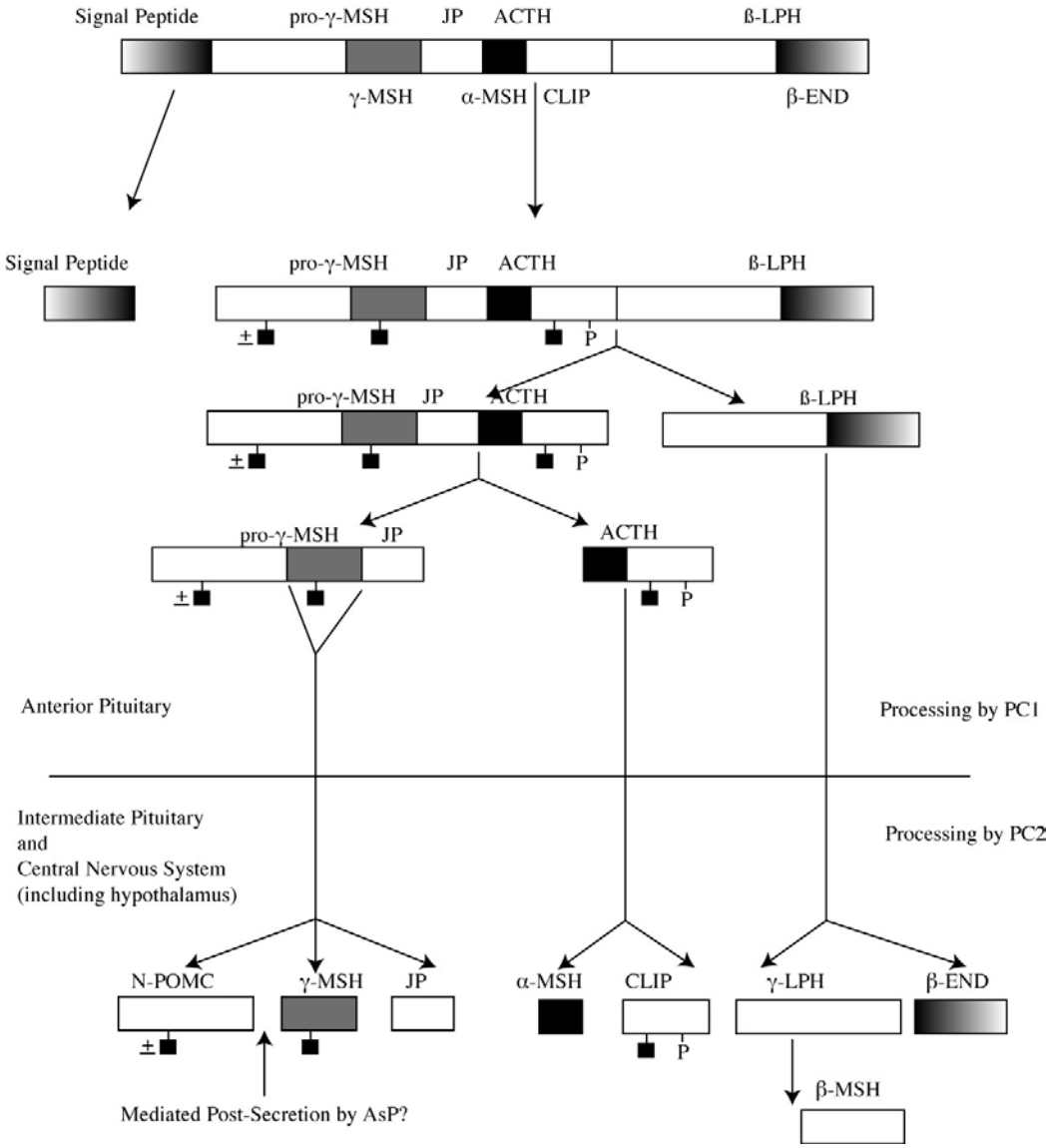

Figure 2. General depiction of consensus preproopiomelanocortin (pre-POMC) structure, post-translational processing, and post-translational modifications. Major processing products of the various lobes of the pituitary and central nervous system are noted on the figure. Each peptide is denoted by appreciation (ACTH, adrenocorticotropic hormone; b-LPH, b-lipotrophin; a-, b-, g-MSH, a-, b-, g-melanocyte-stimulating hormone; CLIP, corticotropin-like intermediate lobe peptide; b-END, b-endorphin; JP, joining peptide. The signal peptide is indicated and lines with arrows indicate dibasic amino acid cleavage sites. The Adrenal Secretory Protease (AsP) cleavage is still proposed. Phosphorylation (P) and glycosylation sites (■) are indicated on the figure.

The processing of POMC in the hypothalamus is similar to the intermediate pituitary lobe in that alpha-MSH is the major product due to the presence of both PC1 and PC2. Numerous studies have implicated alpha-MSH as being of prime importance in the regulation of energy balance. Any defects in POMC processing lead to large problems in maintaining energy balance. In humans PC1 plays a critical role in this processing as evidenced by two separate cases where PC1 is deficient. In the first case a woman with severe early onset obesity was found to be a compound heterozygote for PC1 mutations that either resulted in a proteolytically inactive precursor or a frame shift that rendered an incomplete and nonfunctional enzyme (27). In the second case the patient was found to be a compound heterozygote for two loss of function mutations, one mutation being a premature stop codon within the catalytic domain of PC1 and the other a deletion of a highly conserved alamine (213) near the catalytically active histidine 208 residue (28). These two patients greatly contrast with the phenotype of the PC1 knockout mouse, which has several defects but is not obese and in fact is slightly smaller than wild-type mice (29). The PC1 knockout mouse does have defects in producing ACTH, however a later study, using both radioimmunoassay and peptidomic techniques, revealed that there is no decrease in alpha-melanocyte stimulating hormone (MSH) in PC1

knockout mice (30). The apparent differences between the human patients and the knockout mouse suggest that the processing of POMC may vary between mouse and human and also suggests that the genetic background plays a key role in development of such a multivariable phenotype such as obesity. While our understanding of POMC processing is clearly incomplete, these data strongly show that PC2 presence is important in the generation of the anorexigenic peptide alpha-MSH and that PC1 activity alone is needed to generate ACTH.

Recent experimental evidence suggests that POMC processing may not stop upon secretion of peptides from neuroendocrine cells and adds another layer to the complex processing of this important precursor. For some time there has been a hypothesis suggesting that N-POMC fragments cleared of gamma-MSH have mitogenic activity on the adrenal gland while the presumed supporter of adrenal growth and maintenance, ACTH, has no such activity (31-34). This evidence led to the hypothesis that pro-gamma-MSH was cleaved extraceullarly as no N-POMC peptides lacking gamma-MSH are found in the circulation. In 2001 a group found the first evidence that adrenal mitogenesis depended on an adrenal protease activity (35). Reasoning that a trypsin or serine like protease activity was necessary for cleaving pro-gamma-MSH due to *in vitro* evidence that trypsinization resulted in N-POMC peptides that stimulate adrenal growth, the investigators injected aprotinin (a serine protease inhibitor) into groups of rats prior to unilateral adrenalectomy. This results in a dramatic decrease in adrenal weight compared with controls. In addition aprotinin inhibits the growth of a mouse adrenocortical tumor cell line (35). Utilizing degenerate PCR for a histidine/asparginine/serine active site, an adrenal cDNA library was screened for novel proteases. One such protease was cloned and dubbed the adrenal secretory protease (AsP) due to the fact that it possesses a secretory signal. AsP is found extracellularly on the proliferative adrenal cortex and can cleave its proposed substrate pro-γ-MSH (35). In follow up work a potential adrenal receptor for N-POMC peptides has been isolated (36). These data suggest that POMC processing can continue post-secretion from tissues and highlights the importance of fully understanding the processing of a prohormone in order to understand the physiological roles of the peptides derived from it.

5. PROTHYROTROPIN RELEASING HORMONE (proTRH)

Thyrotropin releasing hormone (TRH) was first discovered by two independent research groups and was one of the founding discoveries of the field of neuroendocrinology (37, 38). Both groups demonstrated that isolated TRH was responsible for release of thyroid stimulating hormone (TSH) from the pituitary and determined the molecular structure of this three-amino acid hormone. This required Herculean biochemical efforts by these groups, utilizing millions of hypothalami from either pigs or sheep, which was described by Guillemin in a recent review (39). TRH is a central regulator of the hypothalamic-pituitary-thyroid axis and is thus partially responsible for the biosynthesis and release of thyroid hormone through its regulation of TSH.

Our laboratory has done much of the work on the processing of this important prohormone. In the course of our studies we have found that PC1 and PC2 are the enzymes that primarily process proTRH in the hypothalamus and that the ordering of proTRH processing is dependent on an initial PC1 cleavage at one of two sites (Figure 3; 40-45). While the processing for the hypothalamus remains well understood, proTRH is expressed in numerous brain regions and other tissues such as pancreas where the processing is not as well studied.

Recent interest in TRH has been intense due to the fact that the hypothalamic-pituitary-thyroid axis is now recognized to be a central player in weight control and energy balance. Several findings from our lab implicate TRH in this process. First and foremost is the finding that leptin, a hormone produced by adipocytes that is known to regulate the accumulation of fat deposits, regulates preproTRH gene expression and TRH release *in vitro* (46). Following this others have shown that TRH is regulated directly by leptin *in vivo* (47, 48). Additionally, our laboratory found that administration of leptin to starved animals stimulates proTRH biosynthesis and also simultaneously results in up regulation of the convertases involved in processing the proTRH precursor into bioactive peptides (49). TRH then seems to be an important player in energy balance.

Hypothalamic TRH is widely reported to play an important role in the thermoregulatory response of mammals to a cold environment (50). In laboratory tests animals are typically subjected to cold room conditions (temperatures varying between 4-7^{0}C) for experimental time periods and core body temperatures are measured before, during, and after cold stress and compared to the temperature of control animals. Under such experimental situations TRH$^{-/-}$ mice have been reported to have problems with thermoregulation when faced with cold stress (51). This supports results from our laboratory group in collaboration with Dr. Bill Wetsel of Duke University in which we observed that CPE$^{fat/fat}$ mice had defects in normal TRH production and processing and that these mice could not properly thermoregulate their body temperature when exposed to cold stress (52). This study gives indirect evidence of a link between TRH and thermoregulation but such a connection is supported by the work reported with TRH$^{-/-}$ mice.

Initially TRH was the only peptide derived from proTRH known to behave as a hormone but recent work has demonstrated that the other peptides derived from proTRH may have physiological function. Rats undergoing suckling have increased levels of preproTRH177-199 (pFE22) in the paraventricular nucleus of the hypothalamus (53), and pFE22 has been found to inhibit dopamine neurons from the arcuate nucleus that in turn reduce the level of prolactin in the circulation (Goldstein, JMN,31,1-14 in press). Additionally, rats experiencing morphine withdrawal have altered levels of different proTRH-derived peptides in

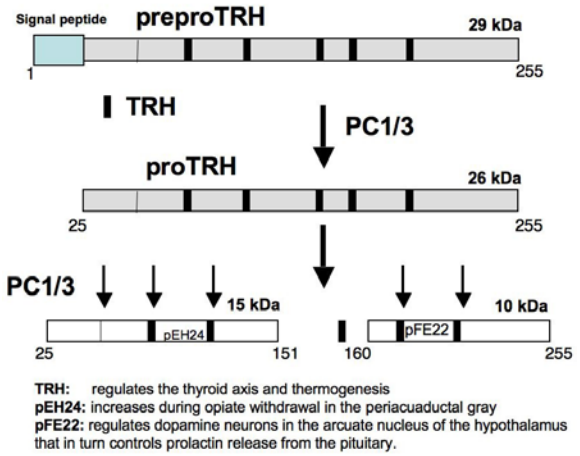

Figure 3. General depiction of rat prothyrotropin-releasing-hormone (proTRH) structure and post-translational processing. Arrows indicate dibasic sites where PCs endoproteolyze proTRH. TRH moieties are represented as black bars.

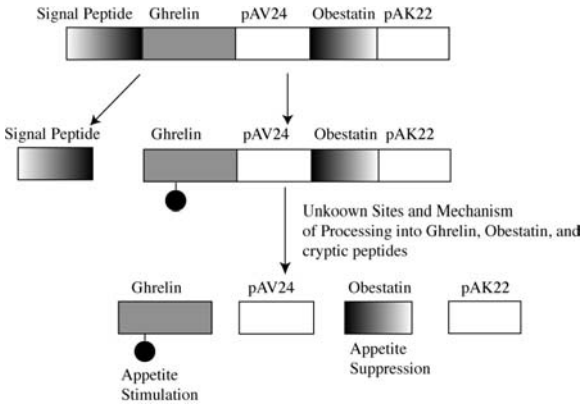

Figure 4. General depiction of human preproGhrelin structure and post-translational processing, and post-translational modifications. The mechanism, sites, and enzymes involved with preproGhrelin processing are not currently known, but it is known that Ghrelin and Obestatin are formed and possess biological activity. Acylation (●) is indicated on the figure.

various regions of the brain suggesting a role for the involvement of proTRH-derived peptides in morphine withdrawal (54). Another proTRH-derived peptide, pST10 has been found to potentiate gastric acid signaling and to have a specific receptor (55).

Morphological and processing studies from other labs also suggest that TRH may not be the only functional peptide derived from proTRH. Indeed an earlier immunohistochemical study revealed that in the thalamic reticular nucleus (a brain region containing GABAnergic neurons that abuts the thalamus and is of incompletely understood function) and other brain regions, no TRH is detected but other proTRH peptides are present (56). In an extensive study utilizing *in situ* hybridization histochemistry the localization of proTRH, PC1, and PC2 was determined (57). Interestingly, in several brain regions proTRH colocalizes with only PC1 or PC2, in the hypothalamus proTRH, PC1, and PC2 colocalize; and in the thalamic reticular nucleus proTRH is present but both PC1 and PC2 were not detectable (which supports the earlier finding of only non-TRH peptides being present in this tissue). These studies suggest that proTRH is differentially processed in various brain regions. In light of such results it is unlikely that the peptide sequences residing between TRH residues in proTRH are simply present to guide delivery of TRH to the appropriate cellular compartments and that proTRH may produce peptides with multiple functions like POMC.

6. GHRELIN AND OBESTATIN

Energy balance and food intake are regulated by a complex set of peptides that originate from multiple tissues. The hypothalamus has been understood to play an important role in this process and the discovery of leptin and its roles have shed new light on the role of adipose tissue as an endocrine organ. Recently the gut peptide ghrelin (Figure 4) has lead to a better understanding of how satiety signals are relayed from the gut to the hypothalamus. ghrelin is a 28 amino acid acylated peptide that was initially discovered due to its observed binding to the growth hormone secretagogue receptor (GHS-R) and stimulation of release of growth hormone from the pituitary (58). Less than a year after ghrelins discovery, evidence was published that implicated Ghrelin as a regulator of energy balance (59). In this study the authors found that the GSH-R is present in the hypothalamus, that intracerbroventricular administration of ghrelin increased food intake and body weight, and that ghrelin levels increased in response to fast and decreased in response to feeding. ghrelin antagonizes leptins effects independent of growth hormone, and seems to do so by primarily activating neuropeptide Y (NPY) neurons in the hypothalamus (60, 61). Research on ghrelin remains intensive, as this peptide hormone appears to play an important role in energy balance.

While much of the work on ghrelin has focused on the mature acylated peptide secreted from the gut work on its complete biosynthesis is not fully understood. A recent study has shown that the story surrounding ghrelin may be more complicated than first thought (62). Utilizing a bioinformatics search, another putative peptide in pro-ghrelin was found based on the high degree of sequence conservation among 11 mammals, the presence of a conserved carboxy-terminal glycine residue that could serve as a site of amidation, and the fact that the sequence is flanked by conserved basic residues that could serve as the sites of PC action. Utilizing the known sequence data the authors generated an antibody against a synthetic peptide comprising the newfound sequence, which they dubbed obestatin. Utilizing radioimmunoassay they found that obestatin was indeed present in the gut. Two obestatin immunoreactive peaks were identified when purified with the first corresponding to the predicted obestatin and the second to a processed form of obestatin, suggesting that the

peptide can undergo further processing. A synthetic amidated form of obestatin administered intracerebroventicularly to rats caused weight loss and decreased feeding, exactly opposite to the effect of ghrelin. In addition, the orphan G-protein coupled receptor GPR39 was identified as the putative receptor for obestatin. Unexpectedly for a peptide regulating feeding, the serum levels of obestatin did not change when the feeding state of the animal was changed from fed to fasting or vice versa. However, additional research by separate research groups has called the findings on obestatin into question (63). Nonetheless, these highly interesting results reveal that proghrelin is capable of producing two peptides with potentially antagonistic action that have their own distinct receptors and revealed a previously unidentified peptide through bioinformatics and processing analysis. This work highlights the importance of fully studying the processing of prohormones. Future processing studies may reveal how the levels of the ghrelin and obestatin are independently regulated while being derived from the same prohormone and may more clearly determine the role of obestatin in energy balance..

7. SEARCHING FOR NEW PEPTIDES

The example of the bioinformatic discovery of obestatin highlights the importance of continuing to use new tools to search for biologically important peptides. This example highlights the fact that unidentified functional peptides may be residing within already discovered precursor molecules. While bioinformatics provide an essential predictive tool that can guide the search for new peptides faster than the classical bioassay approach, computer analysis cannot reveal if such peptides are actually synthesized or processed *in vivo*. The field of proteomics holds great promise as means to find new peptides as well as for a way to better study expression levels of known peptides under physiological conditions.

One such proteomic technique pioneered by Lloyd Fricker's group takes advantage of the processing defects of the $CPE^{fat/fat}$ mouse to isolate peptides that have not had dibasic residues removed from their carboxy - terminal tails (64). These peptides are purified with an anhydrotrypsin affinity column, which binds the dibasic extended peptides allowing for a more specific purification of neuropeptides as opposed to other peptides, which abound in biological samples due to degradation of proteins. Recently Fricker's group has refined their techniques further and shown that comparative analysis with wild-type mice can result in the discovery of unknown peptides (11). Fricker's group has recently written an excellent review highlighting their methodologies and others used in the search for novel peptides (65).

8. CONCLUSION

Prohormone precursors possessing multiple functional peptides highlight the need to fully understand the processing and secretion of all the peptides derived from a prohormone. This is particularly highlighted by the discovery that proGhrelin produces not only Ghrelin put the Ghrelin antagonist Obestatin as well. Additionally, our work on proTRH highlights the potential physiologic activity of non-TRH peptides supported as well by the finding that certain brain regions produce non-TRH peptides and no TRH. POMC produces several important peptides and recent work on the post-secretion processing of N-POMC peptides highlights the importance of continuing to study the processing of even well characterized prohormones. What each of these examples highlights is that the post-translational regulation of prohormone precursors allows for another layer of regulatory control on peptide secretion and physiological regulation. The discovery of obestatin points to the need to fully characterize precursor protein processing and the fact that many important peptides remain unidentified.

9. ACKNOWLEDGMENT

This study were supported by NIDDK/NIH grant R01 DK58148 and R01 NINDS/NIH grant NS045231 to EAN.

10. REFERENCES

1. Steiner DF, C. D., Spigelman L, Aten B: Insulin biosynthesis: evidence for a precursor. *Science*, 157, 697-700 (1967)
2. Smeekens, S. P. & D. F. Steiner: 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)
3. Seidah, N. G. & A. Prat: Precursor convertases in the secretory pathway, cytosol and extracellular milieu. *Essays Biochem*, 38, 79-94 (2002)
4. Zhou, A., G. Webb, X. Zhu & D. F. Steiner: Proteolytic processing in the secretory pathway. *J Biol Chem*, 274, 20745-8 (1999)
5. Smeekens, S. P., S. J. Chan & D. F. Steiner: The biosynthesis and processing of neuroendocrine peptides: identification of proprotein convertases involved in intravesicular processing. *Prog Brain Res*, 92, 235-46 (1992)
6. Smeekens, S. P. & D. F. Steiner: Processing of peptide precursors. Identification of a new family of mammalian proteases. *Cell Biophys*, 19, 45-55 (1991)
7. Fan, X., S. J. Olson & M. D. Johnson: Immunohistochemical localization and comparison of carboxypeptidases D, E, and Z, alpha-MSH, ACTH, and MIB-1 between human anterior and corticotroph cell "basophil invasion" of the posterior pituitary. *J Histochem Cytochem*, 49, 783-90 (2001)
8. Fan, X., S. Spijker, D. B. Akalal & G. T. Nagle: Neuropeptide amidation: cloning of a bifunctional alpha-amidating enzyme from Aplysia. *Brain Res Mol Brain Res*, 82, 25-34 (2000)
9. Varlamov, O., F. J. Eng, E. G. Novikova & L. D. Fricker: Localization of metallocarboxypeptidase D in AtT-20 cells. Potential role in prohormone processing. *J Biol Chem*, 274, 14759-67 (1999)
10. Yasothornsrikul, S., T. Toneff, S. R. Hwang & V. Y. Hook: Arginine and lysine aminopeptidase activities in

chromaffin granules of bovine adrenal medulla: relevance to prohormone processing. *J Neurochem*, 70, 153-63 (1998)
11. Lim, J., I. Berezniuk, F. Y. Che, R. Parikh, R. Biswas, H. Pan & L. D. Fricker: Altered neuropeptide processing in prefrontal cortex of Cpe mice: implications for neuropeptide discovery. *J Neurochem* (2006)
12. Prigge, S. T., R. E. Mains, B. A. Eipper & L. M. Amzel: New insights into copper monooxygenases and peptide amidation: structure, mechanism and function. *Cell Mol Life Sci*, 57, 1236-59 (2000)
13. Prigge, S. T., A. S. Kolhekar, B. A. Eipper, R. E. Mains & L. M. Amzel: Amidation of bioactive peptides: the structure of peptidylglycine alpha-hydroxylating monooxygenase. *Science*, 278, 1300-5 (1997)
14. Kolhekar, A. S., M. S. Roberts, N. Jiang, R. C. Johnson, R. E. Mains, B. A. Eipper & P. H. Taghert: Neuropeptide amidation in Drosophila: separate genes encode the two enzymes catalyzing amidation. *J Neurosci*, 17, 1363-76 (1997)
15. Husten, E. J. & B. A. Eipper: Purification and characterization of PAM-1, an integral membrane protein involved in peptide processing. *Arch Biochem Biophys*, 312, 487-92 (1994)
16. Eipper, B. A., D. A. Stoffers & R. E. Mains: The biosynthesis of neuropeptides: peptide alpha-amidation. *Annu Rev Neurosci*, 15, 57-85 (1992)
17. Mains, R. E., B. A. Eipper & N. Ling: Common precursor to corticotropins and endorphins. *Proc Natl Acad Sci U S A*, 74, 3014-8 (1977)
18. Nakanishi, S., A. Inoue, T. Kita, M. Nakamura, A. C. Chang, S. N. Cohen & S. Numa: Nucleotide sequence of cloned cDNA for bovine corticotropin-beta-lipotropin precursor. *Nature*, 278, 423-7 (1979)
19. Bertagna, X., F. Camus, F. Lenne, F. Girard & J. P. Luton: Human joining peptide: a proopiomelanocortin product secreted as a homodimer. *Mol Endocrinol*, 2, 1108-14 (1988)
20. Bertagna, X., F. Lenne, D. Comar, J. F. Massias, H. Wajcman, V. Baudin, J. P. Luton & F. Girard: Human beta-melanocyte-stimulating hormone revisited. *Proc Natl Acad Sci U S A*, 83, 9719-23 (1986)
21. Bertagna, X., D. Seurin, L. Pique, J. P. Luton, H. Bricaire & F. Girard: Peptides related to the NH2-terminal end of proopiocortin in man. *J Clin Endocrinol Metab*, 56, 489-95 (1983)
22. Takumi, I., D. F. Steiner, N. Sanno, A. Teramoto & R. Y. Osamura: Localization of prohormone convertases 1/3 and 2 in the human pituitary gland and pituitary adenomas: analysis by immunohistochemistry, immunoelectron microscopy, and laser scanning microscopy. *Mod Pathol*, 11, 232-8 (1998)
23. Winsky-Sommerer, R., S. Benjannet, C. Rovere, P. Barbero, N. G. Seidah, J. Epelbaum & P. Dournaud: Regional and cellular localization of the neuroendocrine prohormone convertases PC1 and PC2 in the rat central nervous system. *J Comp Neurol*, 424, 439-60 (2000)
24. Seidah, N. G., M. Marcinkiewicz, S. Benjannet, L. Gaspar, G. Beaubien, M. G. Mattei, C. Lazure, M. Mbikay & M. Chretien: Cloning and primary sequence of a mouse candidate prohormone convertase PC1 homologous to PC2, Furin, and Kex2: distinct chromosomal localization and messenger RNA distribution in brain and pituitary compared to PC2. *Mol Endocrinol*, 5, 111-22 (1991)
25. Laurent, V., L. Jaubert-Miazza, R. Desjardins, R. Day & I. Lindberg: Biosynthesis of proopiomelanocortin-derived peptides in prohormone convertase 2 and 7B2 null mice. *Endocrinology*, 145, 519-28 (2004)
26. Miller, R., W. Aaron, T. Toneff, D. Vishnuvardhan, M. C. Beinfeld & V. Y. Hook: Obliteration of alpha-melanocyte-stimulating hormone derived from POMC in pituitary and brains of PC2-deficient mice. *J Neurochem*, 86, 556-63 (2003)
27. Jackson, R. S., J. W. Creemers, S. Ohagi, M. L. Raffin-Sanson, L. Sanders, C. T. Montague, J. C. Hutton & S. O'Rahilly: Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet*, 16, 303-6 (1997)
28. Jackson, R. S., J. W. Creemers, I. S. Farooqi, M. L. Raffin-Sanson, A. Varro, G. J. Dockray, J. J. Holst, P. L. Brubaker, P. Corvol, K. S. Polonsky, D. Ostrega, K. L. Becker, X. Bertagna, J. C. Hutton, A. White, M. T. Dattani, K. Hussain, S. J. Middleton, T. M. Nicole, P. J. Milla, K. J. Lindley & S. O'Rahilly: Small-intestinal dysfunction accompanies the complex endocrinopathy of human proprotein convertase 1 deficiency. *J Clin Invest*, 112, 1550-60 (2003)
29. Zhu, X., A. Zhou, A. Dey, C. Norrbom, R. Carroll, C. Zhang, V. Laurent, I. Lindberg, R. Ugleholdt, J. J. Holst & D. F. Steiner: Disruption of PC1/3 expression in mice causes dwarfism and multiple neuroendocrine peptide processing defects. *Proc Natl Acad Sci U S A*, 99, 10293-8 (2002)
30. Pan, H., D. Nanno, F. Y. Che, X. Zhu, S. R. Salton, D. F. Steiner, L. D. Fricker & L. A. Devi: Neuropeptide processing profile in mice lacking prohormone convertase-1. *Biochemistry*, 44, 4939-48 (2005)
31. Estivariz, F. E., F. Iturriza, C. McLean, J. Hope & P. J. Lowry: Stimulation of adrenal mitogenesis by N-terminal proopiocortin peptides. *Nature*, 297, 419-22 (1982)
32. Hornsby, P. J.: Regulation of adrenocortical cell proliferation in culture. *Endocr Res*, 10, 259-81 (1984)
33. Jackson, S., P. Salacinski, J. Hope & P. J. Lowry: An investigation of N-terminal pro-opiocortin peptides in the rat pituitary. *Peptides*, 4, 431-8 (1983)
34. Lowry, P. J., L. Silas, C. McLean, E. A. Linton & F. E. Estivariz: Pro-gamma-melanocyte-stimulating hormone cleavage in adrenal gland undergoing compensatory growth. *Nature*, 306, 70-3 (1983)
35. Rao, A. J., J. A. Long & J. Ramachandran: Effects of antiserum to adrenocorticotropin on adrenal growth and function. *Endocrinology*, 102, 371-8 (1978)
36. Bicknell, A. B., K. Lomthaisong, R. J. Woods, E. G. Hutchinson, H. P. Bennett, R. T. Gladwell & P. J. Lowry: Characterization of a serine protease that cleaves pro-gamma-melanotropin at the adrenal to stimulate growth. *Cell*, 105, 903-12 (2001)
37. Bicknell, A. B.: Identification of a receptor for N-POMC peptides. *Endocr Res*, 28, 309-14 (2002)
38. Burgus, R., T. F. Dunn, D. Desiderio, W. Vale & R. Guillemin: [Synthetic polypeptide derivatives with TRF hypophysiotropic activity. New data]. *C R Acad Sci Hebd Seances Acad Sci D*, 269, 226-8 (1969)

39. Boler, J., F. Enzmann, K. Folkers, C. Y. Bowers & A. V. Schally: The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidyl-proline amide. *Biochem Biophys Res Commun*, 37, 705-10 (1969)
40. Guillemin, R.: Hypothalamic hormones a.k.a. hypothalamic releasing factors. *J Endocrinol*, 184, 11-28 (2005)
Mulcahy, L. R., C. A. Vaslet & E. A. Nillni: Prohormone-convertase 1 processing enhances post-Golgi sorting of prothyrotropin-releasing hormone-derived peptides. *J Biol Chem*, 280, 39818-26 (2005)
41. Schaner, P., R. B. Todd, N. G. Seidah & E. A. Nillni: Processing of prothyrotropin-releasing hormone by the family of prohormone convertases. *J Biol Chem*, 272, 19958-68 (1997)
42. Nillni, E. A., L. G. Luo, I. M. Jackson & P. McMillan: Identification of the thyrotropin-releasing hormone precursor, its processing products, and its coexpression with convertase 1 in primary cultures of hypothalamic neurons: anatomic distribution of PC1 and PC2. *Endocrinology*, 137, 5651-61 (1996)
43. Cruz, I. P. & E. A. Nillni: Intracellular sites of prothyrotropin-releasing hormone processing. *J Biol Chem*, 271, 22736-45 (1996)
44. Nillni, E. A., K. A. Sevarino & I. M. Jackson: Processing of proTRH to its intermediate products occurs before the packing into secretory granules of transfected AtT20 cells. *Endocrinology*, 132, 1271-7 (1993)
45. Nillni, E. A., K. A. Sevarino & I. M. Jackson: Identification of the thyrotropin-releasing hormone-prohormone and its posttranslational processing in a transfected AtT20 tumoral cell line. *Endocrinology*, 132, 1260-70 (1993)
46. Nillni, E. A., C. Vaslet, M. Harris, A. Hollenberg, C. Bjorbak & J. S. Flier: Leptin regulates prothyrotropin-releasing hormone biosynthesis. Evidence for direct and indirect pathways. *J Biol Chem*, 275, 36124-33 (2000)
47. Harris, M., C. Aschkenasi, C. F. Elias, A. Chandrankunnel, E. A. Nillni, C. Bjoorbaek, J. K. Elmquist, J. S. Flier & A. N. Hollenberg: Transcriptional regulation of the thyrotropin-releasing hormone gene by leptin and melanocortin signaling. *J Clin Invest*, 107, 111-20 (2001)
48. Guo, F., K. Bakal, Y. Minokoshi & A. N. Hollenberg: Leptin signaling targets the thyrotropin-releasing hormone gene promoter *in vivo*. *Endocrinology*, 145, 2221-7 (2004)
49. Sanchez, V. C., J. Goldstein, R. C. Stuart, V. Hovanesian, L. Huo, H. Munzberg, T. C. Friedman, C. Bjorbaek & E. A. Nillni: Regulation of hypothalamic prohormone convertases 1 and 2 and effects on processing of prothyrotropin-releasing hormone. *J Clin Invest*, 114, 357-69 (2004)
50. Arancibia, S., F. Rage, H. Astier & L. Tapia-Arancibia: Neuroendocrine and autonomous mechanisms underlying thermoregulation in cold environment. *Neuroendocrinology*, 64, 257-67 (1996)
51. Ozawa A, Y. M., Hashida T, Shibusawa N, Hosoya T, Monden T, Satoh T, Mori M: Roles of TRH in the regulation of core temperature under cold exposure: Analysis of TRH-knockout mice. *Folia Endocrinologica Japonica*, 75, Abstract #0029-0661 (1999)
52. Nillni, E. A., W. Xie, L. Mulcahy, V. C. Sanchez & W. C. Wetsel: Deficiencies in pro-thyrotropin-releasing hormone (pro-TRH) processing and abnormalities in thermoregulation in Cpefat/fat mice. *J Biol Chem* (2002)
53. Nillni, E. A., F. Aird, N. G. Seidah, R. B. Todd & J. I. Koenig: PreproTRH (178-199) and two novel peptides (pFQ7 and pSE14) derived from its processing, which are produced in the paraventricular nucleus of the rat hypothalamus, are regulated during suckling. *Endocrinology*, 142, 896-906 (2001)
54. Nillni, E. A., A. Lee, G. Legradi & R. M. Lechan: Effect of precipitated morphine withdrawal on post-translational processing of prothyrotropin releasing hormone (proTRH) in the ventrolateral column of the midbrain periaqueductal gray. *J Neurochem*, 80, 874-84 (2002)
55. Yang, H. & Y. Tache: Prepro-TRH- (160-169) potentiates gastric acid secretion stimulated by TRH microinjected into the dorsal motor nucleus of the vagus. *Neurosci Lett*, 174, 43-6 (1994)
56. Lechan, R. M., P. Wu & I. M. Jackson: Immunocytochemical distribution in rat brain of putative peptides derived from thyrotropin-releasing hormone prohormone. *Endocrinology*, 121, 1879-91 (1987)
57. Pu, L. P., W. Ma, J. L. Barker & Y. P. Loh: Differential coexpression of genes encoding prothyrotropin-releasing hormone (pro-TRH) and prohormone convertases (PC1 and PC2) in rat brain neurons: implications for differential processing of pro-TRH. *Endocrinology*, 137, 1233-41 (1996)
58. Kojima, M., H. Hosoda, Y. Date, M. Nakazato, H. Matsuo & K. Kangawa: Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, 402, 656-60 (1999)
59. Tschop, M., D. L. Smiley & M. L. Heiman: Ghrelin induces adiposity in rodents. *Nature*, 407, 908-13 (2000)
60. Nakazato, M., N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa & S. Matsukura: A role for ghrelin in the central regulation of feeding. *Nature*, 409, 194-8 (2001)
61. Shintani, M., Y. Ogawa, K. Ebihara, M. Aizawa-Abe, F. Miyanaga, K. Takaya, T. Hayashi, G. Inoue, K. Hosoda, M. Kojima, K. Kangawa & K. Nakao: Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes*, 50, 227-32 (2001)
62. Zhang, J. V., P. G. Ren, O. Avsian-Kretchmer, C. W. Luo, R. Rauch, C. Klein & A. J. Hsueh: Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science*, 310, 996-9 (2005)
63. Nogueiras R, Pfluger P, Tovar S, Arnold M, Mitchell S, Morris A, Perez-Tilve D, Vazquez MJ, Wiedmer P, Castaneda TR, DiMarchi R, Tschop M, Schurmann A, Joost HG, Williams LM, Langhans W, Dieguez C: Effects of obestatin on energy balance and growth hormone secretion in rodents. Endocrinology, 148(1), 21-6 (2007)
64. Che, F. Y., L. Yan, H. Li, N. Mzhavia, L. A. Devi & L. D. Fricker: Identification of peptides from brain and pituitary of Cpe (fat)/Cpe (fat) mice. *Proc Natl Acad Sci U S A*, 98, 9971-6 (2001)
65. Fricker, L. D., J. Lim, H. Pan & F. Y. Che: Peptidomics: Identification and quantification of

endogenous peptides in neuroendocrine tissues. *Mass Spectrom Rev*, 25, 327-44 (2006)

Abbreviations: PC, Prohormone Convertase; CP, Carboxypeptidase; PAM, Peptidyl Glycine alpha-amidating monoxygenase; ACTH, Adrenocorticotrophin; POMC, Proopiomelanocortin; JP, Joining Peptide; AsP, Adrenal Secretory Protease; TRH, Thyrotropin Releasing Hormone; TSH, Thyroid Stimulating Hormone; GHS-R, Growth Hormone Secretagogue Receptor; NPY, Neuropeptide Y; MSH, Melanocyte Stimulating Hormone; LPH, Lipotropic Hormone; CLIP, corticotropin-like intermediate lobe peptide.

Key Words: Energy Balance, Prohormone, Proopiomelanocortin, Prothyrotropin Releasing Hormone, Ghrelin, Endocrinology, Processing, Hormone, Neuropeptide, Review

Send correspondence to: Dr Eduardo A. Nillni, Brown Medical School/Rhode Island Hospital, Division of Endocrinology, Pierre Galletti Building, 55 Claverick Street, 3 floor/Room 320, Providence, RI 02903, Tel: 401 444-5733, Fax: 401 444-696, E-mail: Eduardo_Nillni@Brown.edu

http://www.bioscience.org/current/vol12.htm