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Neuropeptide PEN and Its Receptor GPR83: Distribution, Signaling, and Regulation

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Abstract

Neuropeptides are chemical messengers that act to regulate a number of physiological processes, including feeding, reward, pain, and memory, among others. PEN is one of the most abundant hypothalamic neuropeptides; however, until recently, its target receptor remained unknown. In this Review, we summarize recent developments in research focusing on PEN and its receptor GPR83. We describe the studies leading to the deorphanization of GPR83 as the receptor for PEN. We also describe the signaling mediated by the PEN-GPR83 system, as well as the physiological roles in which PEN-GPR83 has been implicated. As studies have suggested a role for the PEN-GPR83 system in food intake and body weight regulation, as well as in drug addiction and reward disorders, a thorough understanding of this novel neuropeptide—receptor system will help identify novel therapeutic targets to treat pathophysiological conditions involving PEN-GPR83.

Graphical Abstract

Keywords

G protein-coupled receptor; de-orphanization; neuropeptide processing; hypothalamic signaling; feeding behaviors; drug addiction

Author Contributions

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INTRODUCTION TO THE PEN-GPR83 SYSTEM

Neuropeptides are peptide neuromodulators that are expressed by neurons and act on neural targets by activating neuropeptide receptors.¹ These receptors serve as therapeutic targets for the treatment of a number of pathophysiological conditions, including obesity, pain, and addiction.^{2–6} Major technological advances in the 1980s involving improved sensitivity of peptide purification methods and single-neuron mRNA sequencing techniques have led to an explosion in the number of newly discovered neuropeptides.⁷ However, to date, receptors for many of these neuropeptides remain largely unknown. In addition, currently there are a number of “orphan” G protein-coupled receptors (GPCRs) for which endogenous ligands or function are yet to be identified.^{8,9} These “orphan” neuropeptides and GPCRs represent an untapped resource for the development of therapeutics to treat chronic diseases including drug abuse disorders, obesity, and neuropathic pain. With this in mind, much effort has been put toward de-orphanizing these “orphan” peptides/GPCRs. Here, we summarize current insights into one such relationship: that between the neuropeptide PEN and the GPCR named GPR83. This intriguing system promises to deepen our current understanding of neuropeptide/GPCR systems, but also highlights the necessity for further technological advances in order to fully understand its complexity.

Introduction to GPR83.

GPR83 (also known as GPR72, JP05, and GIR) was first identified as a receptor that was upregulated in murine thymoma cells in response to the glucocorticoid dexamethasone; this led to the receptor being named the Glucocorticoid Induced Receptor (GIR).^{10–12} Later studies showed that GPR83 exhibits robust expression in mouse and human brains.^{13,14} In humans, GPR83 is encoded by the *Gpr83* gene, located in the q21 region of chromosome 11.¹⁵ This gene encodes for a 423 amino acid long protein (~48 kDa). GPR83 belongs to the rhodopsin-like class A GPCR family^{16–18} and shares the highest sequence similarity with members of the Neuropeptide Y Receptor family.^{17,19} Although only one isoform of GPR83 has been discovered in humans, up to four isoforms have been identified in mice.^{11,15,20,21} Isoform 1 corresponds to the GPR83 expressed in humans. Isoform 2 has a deletion in exon 2 and is predicted to be nonfunctional, as it lacks the third transmembrane domain. Isoform 3 includes an insertion of 68 amino acids in the second cytoplasmic loop, while Isoform 4 includes an insertion of 20 amino acids in the second cytoplasmic loop.²⁰ The functionality of the isoforms has not been fully studied; however, one study has shown that mice treated with T-cells overexpressing GPR83 isoform 4 (but not isoform 1) exhibited a reduced contact hypersensitivity reaction (an in vivo assay of cell-mediated immune function).²⁰ Most studies with GPR83 focus on isoform 1 from the mouse brain, which is the most highly expressed of all the isoforms and corresponds to the only variant expressed in the human brain.^{22–25} Mice with a deletion of GPR83 (by genomic deletion of exons 2 and 3) show altered food intake and stress-induced anxiety,^{22,26} indicating roles for GPR83 in regulation of feeding, stress modulation, and reward behavior; these will be discussed later in this Review.

PEN Is an Endogenous Ligand of GPR83.

Our laboratory identified the neuropeptide PEN as an endogenous ligand for GPR83²⁵ using a strategy that selects neuropeptide—receptor pairs based on the match between expression/distribution of peptide precursors and orphan GPCRs; this strategy led to the successful identification of another neuropeptide, bigLEN, as an endogenous ligand for the orphan G protein-coupled receptor GPR171.²⁷ To identify a receptor for PEN, we first established that a receptor for PEN in the hypothalamus exhibits properties similar to those of a receptor in Neuro2A cells.²⁵ Next, we selected orphan GPCRs highly expressed both in the hypothalamus and in Neuro2A cells and screened them for signaling by PEN. This led us to identify GPR83 as the receptor, since it was necessary and sufficient to elicit signaling by PEN. To test whether GPR83 is sufficient to function as a receptor for PEN, we expressed GPR83 in CHO cells (a cell line that does not express endogenous GPR83) along with a chimeric G16/i3 protein and tested these cells for signaling by PEN (and other proSAAS peptides as negative controls) using an assay that measures increases in intracellular calcium levels.²⁵ We found that PEN is a selective and potent ligand of GPR83.²⁵ PEN did not elicit signaling in cells expressing either GPR19, GPR108, GPR165, or GPR171 or in hypothalamic membranes from GPR83 knockout mice.²⁵ Since the hypothalamus expresses several GPCRs besides GPR83,²⁸ these results with knockout tissue demonstrate that PEN is not a ligand for other hypothalamic GPCRs, indicating a degree of selectivity for GPR83. To test whether GPR83 is necessary for signaling by PEN, we either used Neuro2A cells (a cell line that expresses endogenous GPR83) with reduced expression of GPR83 using shRNA (knockdown) or used tissues from mice lacking GPR83 (knockout); we showed that knockdown leads to reduced binding and signaling by PEN, whereas knockout of GPR83 leads to a loss of binding and signaling by PEN.²⁵ A recent report by another group showed that knocking down or reducing the levels of GPR83 abolished PEN signaling, as measured by decreased transcription of NFAT5.²⁹ This provides additional evidence that PEN functions, indeed, as an endogenous ligand for GPR83.

PEN Is Derived from the Proprotein ProSAAS.

PEN is one of several neuropeptides derived from the processing of the precursor protein, proSAAS.³⁰ ProSAAS is a 26-kDa protein encoded by the *PCSK1N* gene (chromosomal localization Xp11.3 in humans)³⁰ and is widely expressed in a number of species (including humans, mice, and rats). ProSAAS was first identified from a search for novel neuropeptides in Cpe^{fat}/Cpe^{fat} mice that lack carboxypeptidase E (CPE). These mice have an obese phenotype and harbor a mutation that inactivates and sequesters CPE, a peptide processing enzyme in the secretory pathway responsible for cleaving propeptides into bioactive neuropeptides.³¹ Cpe^{fat}/Cpe^{fat} mice were found to have increased levels of a number of C-terminally extended, intermediary, bioactive peptides (likely due to the inactivation of CPE).³⁰ Many of the most abundant intermediary peptides were found to be derived from the same precursor protein, proSAAS (so termed because one of the peptides contained a distinctive Ser-Ala-Ala-Ser motif).³⁰ The N-terminal region of proSAAS is cleaved to yield the peptides GAV, littleSAAS, and KEP, while the C-terminal region yields PEN and BigLEN (see Figure 1).^{32–34} These other proSAAS-derived peptides do not exhibit sequence similarity with PEN; hence, it is not surprising that they do not bind to or signal via GPR83.²⁵

At the time of the discovery of proSAAS, there were no known receptors for any of its derived peptides; however, researchers were able to glean information about the potential physiological roles of proSAAS-derived peptides, mainly by detecting the outcomes of overexpression or knockout of proSAAS. For example, overexpression of proSAAS decreased processing of POMC,³⁰ a precursor hormone whose peptides are implicated in the regulation of feeding, among other functions.³⁵ In addition, peptides derived from proSAAS were found to be potent and selective inhibitors of prohormone convertase 1 (PC1),^{30,36,37} an enzyme involved in the cleavage and processing of neuropeptides.^{38,39} Later studies suggested that the most potent inhibitory sequence was a 10-amino-acid sequence (VLGALLRVKR) located at amino acids 235–244 of proSAAS.⁴⁰ Although all but the last two amino acids in this sequence are a part of full-length PEN, studies with truncated peptides show that PEN itself does not function as an inhibitor of PC1.³⁶ There is also evidence that the N-terminal region of proSAAS acts as a chaperone that prevents aggregation of β -amyloid, a protein found to be abnormally aggregated in Alzheimer's disease.⁴¹ In this context, a number of studies detected decreased levels of proSAAS-derived peptides in the cerebrospinal fluid (CSF) of patients with Alzheimer's disease^{42–44} and frontotemporal dementia,⁴⁵ suggesting that this decrease could contribute to the etiology of Alzheimer's disease. Additional information about the physiological role of proSAAS-derived peptides has come from genetically modified mice. Mice overexpressing proSAAS exhibited obesity when compared to their wild-type counterparts, while lack of proSAAS yielded slightly underweight mice.^{46,47} Interestingly, proSAAS-knockout mice display an anxiety phenotype,⁴⁶ suggesting a role for proSAAS-derived peptides in anxiety and stress modulation. Together, these studies pointed toward a role for proSAAS-derived peptides not only as a regulator of secretory pathway processing but also as a regulator of body weight and food intake.

To date, not much is known about the specific roles of individual proSAAS-derived peptides except for littleSAAS, BigLEN, and, more recently, PEN. Evidence for a role of littleSAAS in the regulation of circadian clocks comes from a study that reported that exogenous application of this peptide delays peak electrical activity of suprachiasmatic nuclei pacemaker cells in a manner similar to how light-mediated cues regulate circadian time.^{48,49} Evidence for a role for bigLEN and PEN in regulating responses associated with food intake and metabolism comes from studies using intracerebral administration of antibodies to proSAAS-derived peptides (bigLEN, PEN, little LEN, or little SAAS).⁵⁰ Data show that fasted mice given anti-PEN antibodies (which sequester PEN) exhibited reduced food intake for 14 h following antibody injection (compared with 2 h for mice given anti-bigLEN antibodies).⁵⁰ Administration of anti-littleLEN or anti-littleSAAS antibodies had no effect on food intake in these mice.⁵⁰ The differential roles of these proSAAS-derived peptides (i.e., littleSAAS and PEN) are intriguing, as they provide evidence that peptides derived from the same precursor protein can perform different (albeit related) biological functions.

ANATOMICAL DISTRIBUTION OF PEN-GPR83

GPR83 and ProSAAS Have Overlapping Areas of Expression.

In mice, GPR83 mRNA is expressed in limbic structures in the forebrain, in some regions of the hypothalamus, and in the striatum (particularly the nucleus accumbens), and at relatively low levels in the periphery (except thymus) (see Figure 2.¹⁴). The presence of GPR83 protein in thymus implies a role for the receptor in immunological responses. In this context, studies have shown that GPR83 is selectively upregulated in regulatory T-cells (T-reg), a subset of T-cells that play a role in the prevention of immunological responses to self-antigens, as well as in preventing excessive responses that are harmful to the host.⁵¹ Given that location of the receptor usually suggests a functional role, this would be consistent with a role for GPR83 in food intake, modulation of reward pathways, and immunological responses.

In humans, GPR83 mRNA is highly expressed in the brain and spinal cord, and it is found in lower concentrations in the thymus and other tissues.¹³ Within the human brain, GPR83 exhibits particularly high expression in the hypothalamus, striatum, hippocampus, amygdala, and prefrontal cortex.⁵² This would suggest a role for GPR83 in stress, reward, anxiety, fear, learning, and memory.

The precursor proSAAS is highly expressed in the brain, pituitary, adrenal glands, and pancreas, pointing toward a potential neuropeptide role for proSAAS-derived peptides (see Figure 2).³⁰ Although proSAAS mRNA is widely expressed in the brain, its expression is particularly robust in a few specific brain regions, such as the hypothalamus (classically thought to regulate body homeostasis thirst, hunger, and sleep), the hippocampus (memory formation), and the amygdala (memory processing, decision making, and emotional responses, especially fear, anxiety, and aggression).^{30,50,53,54} From the distribution of proSAAS protein and of peptides generated by its proteolytic cleavage, it is clear that these peptides exhibit neuroendocrine distribution and are poised for neuromodulation in multiple brain regions.^{55,56}

Peptides Derived from ProSAAS Exhibit Differential Subcellular Localization.

As mentioned previously, proSAAS is processed into many potential bioactive peptides, including GAV, PEN, bigSAAS, littleSAAS, bigLEN, and littleLEN (Figure 1).³⁴ An interesting finding is that some of these proSAAS-derived peptides exhibit differential subcellular localization; studies in AtT-20 cells (a mouse pituitary tumor cell line) show that littleLEN and PEN localize to the tips of the cell processes, which contain mature secretory vesicles, while bigLEN and PEN-LEN localize to the perinuclear space, a region of the cell associated with the Golgi apparatus, the trans Golgi network (TGN), and other immature secretory vesicles.^{34,57} These data point toward the peptides potentially playing different roles and having distinct functions. An intriguing observation is that peptides generated from proSAAS processing depend on the cellular complement of processing enzymes. In contrast to AtT-20 cells, which generate littleLEN from proSAAS, in PC12 cells (a rat adrenal cell line that does not contain either of the processing enzymes PC1 or PC2), proSAAS is cleaved into bigLEN, PEN, and SAAS, but not littleLEN.^{32,34} This would suggest that the

complement of proSAAS peptides generated depends on the repertoire of specific processing enzymes present in specific brain regions/tissues.

SIGNALING INVOLVING PEN-GPR83

GPR83 Exhibits G_q- and G_i-Mediated Signaling.

Prior to the identification of specific endogenous ligands activating GPR83, information about GPR83 signaling was from studies investigating basal signaling (signaling in the absence of receptor activation).^{23,24,58} These studies showed that the mere presence of GPR83 increased basal levels of accumulation of inositol triphosphate (indicative of G_q-mediated signaling). This was further enhanced by N-terminal truncation of GPR83, supporting the idea that the N-terminal region of GPR83 functions as a negative allosteric modulator of GPR83.^{24,58} Another study showed that the basal activity of GPR83 could be further enhanced by treatment with zinc.^{22,23} Given that neither the presence of GPR83 nor treatment with zinc altered cAMP levels (indicative of either G_i- or G_s-mediated signaling), it was proposed that GPR83 signaled largely through G_q-mediated pathways.^{23,24,58}

Once PEN was identified as a ligand for GPR83, we undertook studies toward a detailed characterization of signaling. Based on previous reports that GPR83 coupled to G_q-proteins, we examined PLC activity in CHO-GPR83 cells (CHO cells overexpressing GPR83) and found that PEN dose-dependently activated PLC with very high potency.²⁵ Because PLC stimulates the production of IP₃ (inositol 1,4,5-triphosphate), which leads to increases in intracellular calcium release, we also examined the effect of PEN on IP₃ levels and intracellular calcium levels.²⁵ Treatment with PEN increased both IP₃ and calcium levels; this is consistent with previous data that GPR83 exhibits G_q-mediated signaling.^{23–25,58} In addition, we found that GPR83 exhibits signaling via G_i activation. Treatment of CHO-GPR83 cells or Neuro2A (which endogenously expresses GPR83) with PEN led to a dose-dependent decrease in cAMP levels.²⁵ This differential signaling via different G proteins appears to be region-specific. For example, in hypothalamic membranes, treatment with PEN led to a robust increase in PLC activity (indicative of G_q-mediated signaling), whereas in hippocampal membranes, PEN treatment led to a significant inhibition of adenylyl cyclase activity (indicative of G_i-mediated signaling); in this latter region PEN had no consistent effects on PLC activity.²⁵ These data suggest that GPR83 can couple to and signal through different G proteins in a region-specific manner. Further studies are needed to explore the implications of the regional effect of PEN on GPR83 signaling and on the mechanisms regulating receptor associations with specific G proteins.

Signaling by PEN-Derived Peptides.

Previous studies have identified a number of PEN-derived peptides in cells and tissues.^{32,33,59,60} An investigation into neuropeptide processing in AtT-20 cells overexpressing proSAAS detected smaller endogenous C-terminally truncated forms of PEN.³² Full-length PEN is 22 amino acids long, while the C-terminally truncated forms include PEN18, PEN19, and PEN20 (see Figure 1).^{32,33} Many of these smaller PEN-derived peptides have also been detected via mass spectrometry in the brains of Cpe^{fat}/Cpe^{fat} mice.^{27,33,61} Given the differential signaling between bigLEN and littleLEN (GPR171 is a receptor for bigLEN

but not littleLEN),²⁷ it is possible that these smaller PEN-derived peptides exhibit differential signaling at GPR83. Additional research is needed to understand how these truncated PEN peptides are generated and in which subcellular compartment. Do these truncated PEN peptides exhibit differential distribution in the brain? If so, how are their levels regulated? Do they bind to GPR83 and/or to other receptors? If they bind to GPR83, do they exhibit signaling similar to or different from PEN? Do they function as GPR83 agonists, antagonists, or biased ligands? Studies addressing these (and other) questions may provide further insights into the regulation of PEN as well as a basis for therapeutics targeting the PEN-GPR83 system.

ROLES OF PEN-GPR83

The PEN-GPR83 System Plays Roles in Regulation of Feeding.

Even before it was identified as a receptor for PEN, GPR83 was thought to play a role in regulation of feeding and reward behavior. When GPR83 was first discovered, it was noted that it shared high sequence similarity with the Neuropeptide Y receptor.¹⁹ Both receptors also display comparable regions of expression,⁶² suggesting similar physiological functions. The similarity between the Neuropeptide Y receptor and GPR83 becomes especially salient once we consider that PEN, the peptide eventually identified as a ligand for GPR83, co-localizes with Neuropeptide Y (and BigLEN) in the hypothalamus.⁵⁰ This suggested that, like the Neuropeptide Y receptor, GPR83 played a role in regulation of feeding and provided the basis for early speculation that NPY might be a ligand for GPR83. In the latter context, although studies have not examined PEN binding to the Neuropeptide Y receptor, one study reported that NPY could bind GPR83,¹⁹ while another found that NPY was able to engage GPR83 only at very high (10 μ M), non-physiological concentrations.²⁵

Other support for the involvement of GPR83 in the regulation of feeding comes from studies showing that it co-localizes with the ghrelin receptor Ghsr1a and with the bigLEN receptor GPR171 in the hypothalamus.^{22,27} As both ghrelin and BigLEN work to regulate appetite and energy expenditure,^{22,27} the colocalization of GPR83 with these receptors lends support to a potential role for GPR83 in food intake regulation. Interestingly, in vitro studies show that GPR83 is in close enough proximity for direct interactions with both the ghrelin receptor Ghsr1a²² and GPR171.²⁷ Moreover, coexpression of GPR83/Ghsr1a or GPR83/GPR171 changed the signaling profiles of the individual receptors.^{22,27} Studies with GPR83 knockout mice show that they do not exhibit increases in body weight when on a high-fat diet.²² Another study reported that decreasing expression of GPR83 in the preoptic area of the hypothalamus caused mice to gain body weight.²¹ Although at first glance, the results of these studies seem to conflict, the first study was performed in GPR83 knockout mice, while the latter study used GPR83 shRNA, resulting in only a partial knockdown of GPR83 localized to the preoptic area of the hypothalamus.²¹ This leaves open the possibility that other brain regions where GPR83 is expressed could contribute to its role in food intake regulation. Further research is necessary to elucidate the mechanisms involved in food intake regulation by GPR83.

The PEN-GPR83 System Is Regulated by Drugs of Abuse.

GPR83 and proSAAS have been reported to be modulated by drugs of abuse. A study showed that expression of GPR83 mRNA was elevated in the prefrontal cortex of mice chronically treated with amphetamines (for at least 7 days) even after amphetamine withdrawal.¹⁰ Sustained activation after exposure to a stressor (e.g., drugs of abuse such as amphetamines) is indicative of proteins that act as regulators of the hypothalamic—pituitary—adrenal (HPA) axis.^{63,64} Drugs of abuse can also modulate the levels of proSAAS-derived peptides in a peptide-specific manner. For example, *Cpe^{fat/fat}* mice chronically treated with morphine for 5 days exhibited decreased expression of bigSAAS intermediary peptides in the hypothalamus, while expression of most proSAAS intermediary peptides remained unchanged.⁶¹ Another study showed that levels of proSAAS-derived peptides are modulated by cocaine treatment.⁶⁵ Wild-type mice treated with cocaine for a 10-day period had decreased levels of proSAAS-derived peptides (namely PEN and littleSAAS) in the nucleus accumbens and ventral tegmental area.⁶⁵ Furthermore, proSAAS-knockout mice treated with cocaine displayed reduced responses to the locomotor effects of cocaine, as well as a lack of sensitization to cocaine.⁶⁵ The same study found that proSAAS-knockout mice treated with amphetamines also displayed reduced responses to the locomotor effects of amphetamines;⁶⁵ this is particularly significant because cocaine and amphetamine have different (although similar and overlapping) mechanisms of action.^{66–68} These data provide further support for the notion that proSAAS-derived peptides can be differentially regulated by drugs of abuse.

A commonality shared by many drugs of abuse is that they are very rewarding; they hyperactivate dopaminergic systems and desensitize them to stimulation by other means.⁶⁹ A common test for this desensitization of the dopaminergic system in mice is the sucrose preference test.^{70,71} In this context, GPR83 knockout mice were found to display increased preference for sucrose, as well as an insensitivity to stress induced by anxiety²⁶ suggestive of a role for the receptor in modulating reward behaviors.²⁶

Stress is a key factor in addiction and reward disorders and particularly in relapse.^{72–75} Both stress and drugs of abuse cause dysregulation of the hypothalamic-pituitary-adrenal axis (HPA axis), which can be assessed in rodents by measuring cortisol (a steroid hormone) levels.^{73–76} Dexamethasone, while not a drug of abuse, is a steroid that is used to mimic changes in cortisol under stressful conditions: acute administration of dexamethasone decreases cortisol levels, while chronic administration leads to increases in cortisol levels. While one study showed that acute administration of dexamethasone increased GPR83 expression in murine thymocytes,¹² another study reported that it decreased GPR83 mRNA expression in the nucleus accumbens, striatum, olfactory tubercle, and hypothalamus.⁷⁷ This suggests a link between GPR83, stress modulation, and addictive behaviors, but further studies are needed to elucidate this potential connection and provide answers to questions raised by the previously mentioned studies. First, what is the effect of chronic dexamethasone administration on the expression of GPR83 in the brain? Do GPR83 knockout mice respond differently to dexamethasone administration? How does chronic stress affect cortisol levels in GPR83 knockout mice, and do they exhibit an increase in self-administration of drugs of abuse after acute/chronic dexamethasone administration?

Answers to these questions will provide key insights into the role of GPR83 in addiction and reward disorders.

DISCUSSION AND CONCLUSION

As research has uncovered novel information about the neuropeptide PEN, it has become increasingly clear just how intricate and complex the PEN-GPR83 system is. The implications of this system are far-reaching, with apparent roles for PEN in food intake regulation, body weight, and reward disorders. The goal of developing therapeutics based on the PEN-GPR83 system will require a thorough understanding of its signaling and regulation in various tissues. In order to further elucidate the complexities of systems such as these, it will be necessary to find ways to monitor the presence of neuropeptides in specific brain regions or the release of neuropeptides at specific sites, and to monitor the effect of the neuropeptide release on behavior. Many questions still remain. Foremost among them: What is the contribution of PEN and of each PEN-derived peptide to the activity of GPR83? Do PEN-derived peptides exhibit distinct brain localization? How can we identify which peptides are actually present in the brain when a particular behavior is observed?

An understanding of the role of PEN will be crucial for any future endeavors involving the PEN-GPR83 system. Tools to identify peptides in individual small brain regions (or, ideally, in individual neurons) would be useful in elucidating the role of each peptide in influencing particular behaviors. Studies to date have used micropunches or regional pools of tissue collected post-mortem to identify peptides via mass spectrometry. While much has been learned using these techniques, it would be ideal if we could identify neuropeptides present in specific brain regions in animals under different behavioral paradigms. Fast-scan voltammetry has previously been used to measure small neurotransmitters such as dopamine and metabolites, and recent studies have demonstrated that these techniques also yield usable data with peptides.⁷⁸

Another major question that remains is whether biased signaling at GPR83 could be exploited to develop therapeutics for treating pathological conditions involving the receptor. Biased signaling has been noted in a number of other GPCRs. Indeed, biased molecules targeting opioid receptors are currently in clinical trials.⁷⁹ While morphine indiscriminately activates both G protein and β -arrestin signaling, new molecules have been synthesized that exhibit biased signaling: they activate G protein-coupled signaling, but do not activate β -arrestin recruitment, thereby bypassing many of the negative side effects associated with morphine as an analgesic (namely tolerance and depression).^{80–82} Preliminary studies indicate that GPR83 exhibits biased signaling; the identification of small molecules that exhibit biased signaling and that modulate behaviors differently will be useful in designing therapeutics for the physiological processes that GPR83 regulates. Another question pertains to the multiple modes of regulation of GPR83. GPR83 has been shown to be regulated by its N-terminus, by ions such as zinc, and by protein–protein interaction with other receptors (e.g., GPR171 or ghrelin receptor Ghsr1a).^{22,23,27} This suggests that GPR83 activation is subject to multiple modes of regulation, leaving open the possibility that there are sites/domains on GPR83 that are responsible for differential modulation. Such studies will serve as the foundation for the identification of small molecules targeting GPR83 that will help

elucidate the role of differential signaling by GPR83 both in normal cell function and in pathological disease states.

Neuropeptide receptors such as GPR83 are potential therapeutic targets for drugs to treat a number of pathophysiological conditions, including food disorders and addiction. Here we describe efforts toward the characterization of this fascinating new novel neuropeptide/GPCR system that has implications in addiction and reward disorders. We find ourselves on the precipice of exciting new frontiers in research and medicine, but with much work still to be done to best understand and utilize this novel system.

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Figure 1.

Proprotein proSAAS is cleaved into a number of bioactive peptides.^{32–34} Furin and CPD cleave BigSAAS from the N-terminal region of proSAAS. BigSAAS is further cleaved into KEP and littleSAAS by enzymes PC1/3, PC2, and CPE. Furin and CPD cleave PEN-LEN from the C-terminal region of proSAAS. PEN-LEN is further processed into PEN and BigLEN by PC1/3 and PC2. BigLEN can be further processed into littleLEN. Recent studies have shown that a number of truncations of PEN are endogenously expressed;^{32,33} the sequences of these forms of PEN are shown here.

Figure 2.

(A) Heatmap of PCSK1N (proSAAS) mRNA distribution in different tissues in mice.

Heatmap was generated with data from the NCBI Web site. (B) Cartoon depicting the subcellular structures that co-localize with proSAAS and proSAAS-derived peptides. Data were obtained from Uniprot. (C) Heatmap of GPR83 mRNA distribution in different tissues in mice. Heatmap was generated with data from the NCBI Web site. (D) Cartoon depicting the subcellular structures that co-localize with GPR83. Data were obtained from Uniprot.