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Targeting the Recently Deorphanized Receptor GPR83 for the Treatment of Immunological, Neuroendocrine and Neuropsychiatric Disorders

Lindsay M. Lueptow, Lakshmi A. Devi¹, and Amanda K. Fakira

Department of Pharmacological Sciences, Freidman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, United States

Abstract

G-protein coupled receptors (GPCRs) are a superfamily of receptors responsible for initiation of a myriad of intracellular signaling cascades. Currently, GPCRs represent approximately 34% of marketed pharmaceuticals, a large portion of which have no known endogenous ligand. These orphan GPCRs represent a large pool of novel targets for drug development. Very recently, the neuropeptide PEN, derived from the proteolytic processing of the precursor proSAAS, has been identified as a selective, high-affinity endogenous ligand for the orphan receptor, GPR83. GPR83 is highly expressed in the brain, spleen and thymus, indicating that this receptor may be a target to treat neurological and immune disorders. In the brain GPR83 is expressed in regions involved in the reward pathway, stress/anxiety responses, learning and memory and metabolism. However, the cell type specific expression of GPR83 in these regions has only recently begun to be characterized. In the immune system, GPR83 expression is regulated by Foxp3 in T-regulatory cells that are involved in autoimmune responses. Moreover, in the brain this receptor is regulated by interactions with other GPCRs, such as the recently deorphanized receptor, GPR171, and other hypothalamic receptors such as MC4R and GHSR. The following review will summarize the properties of GPR83 and highlight its known and potential significance in health and disease, as well as its promise as a novel target for drug development.

1. INTRODUCTION

G-protein coupled receptors (GPCRs) are a superfamily of receptors that convert extracellular signals into intracellular responses, via activation of various G-proteins, critical for a variety of biological functions.¹ Due to the abundance and importance of these receptors, GPCRs represent 34% of pharmaceutical drug targets. Moreover, approximately 15% of GPCRs do not have a known endogenous ligand and are therefore categorized as orphan receptors. These orphan GPCRs represent a large pool of potential therapeutic targets for various disorders.

GPR83 was originally cloned in 1989 and held the status as an orphan receptor until the recent identification of its endogenous ligand, the neuroendocrine peptide PEN, which

Correspondence to: Lindsay M. Lueptow; Amanda K. Fakira.

¹Corresponding author: lakshmi.devi@mssm.edu.

contains a Pro-Glu-Asn tripeptide sequence.²⁻⁴ While progress in understanding the biological role of GPR83 has been slow due to the lack of a known endogenous ligand, the use of shRNA and other viral vectors, as well as GPR83 knockout mice, has allowed some understanding of the role of GPR83 in a number of cellular and behavioral processes.

The initial investigation of GPR83 primarily involved characterization of the expression pattern of GPR83 RNA in organ systems and tissues. Several studies have reported GPR83 in discrete brain regions, such as the hypothalamus, amygdala, hippocampus and striatum, brain areas known to regulate several neurological functions including stress, anxiety, reward and metabolic function. Interestingly, the precursor for peptide PEN (named proSAAS) is also highly expressed in brain regions that express GPR83, such as the hypothalamus, hippocampus and amygdala.⁵⁻¹⁰ GPR83 also is expressed in various immune cells, such as B cells and CD8+ killer T cells, and more specifically, FoxP3+ regulatory T cells, but its role in immune function is not yet fully understood.^{4,11-16} Taken together, these data suggest that the PEN-GPR83 receptor system is of great interest for further study for both affective disorders and immune regulation.

1.1 Discovery of GPR83

GPR83 was originally cloned from murine thymoma WEHI-7TG cells.^{4,11,12} At that time researchers were searching for a glucocorticoid and/or cAMP-inducible gene that mediated lymphocyte cytolysis, in the hope of better understanding the effects of glucocorticoids on T lymphocyte function and cell death. In doing so, they identified clone 4.2, later known as GPR83, as being up-regulated by both the synthetic glucocorticoid dexamethasone, as well as forskolin, an adenylyl cyclase activator.⁴ Because of these findings, GPR83 was named glucocorticoid-induced receptor. However, in the thymoma cells, the time course for GPR83 up-regulation takes around 4 h of exposure to dexamethasone, and researchers have been unable to find any glucocorticoid response elements in the promoter region of the receptor, suggesting that the effect of glucocorticoids on GPR83 expression could be via indirect pathways.^{11,12,17,18}

In 1991, the full sequence of GPR83 was published.¹² Researchers noted a few interesting aspects of the receptor, including the expression of introns in the coding region, which is somewhat unusual for GPCRs. Up to four different GPR83 transcripts have been described, and therefore, likely arise via alternative splicing of these introns.^{11,18,19} Additionally, in two of the transcripts, they found differences in the second cytoplasmic loop, which is often thought to play a role in G-protein-coupling, which suggests that the receptor may be able to differentially couple to different signaling pathways via the structural variation in this region.¹² Interestingly, recent studies by the Devi group demonstrate that GPR83 differentially couples to G-proteins, depending on the brain region. They showed that GPR83 signals through the inhibitory G α_i G-protein in the hippocampus, but G α_q in the hypothalamus.³

While up to four splice variants have been described in mice, the only functional variant described in human brain also happens to be the most highly expressed in the mouse brain, suggesting the use of mouse tissue to study the function of the receptor is appropriate.^{12,18,20} Furthermore, it is unclear if two of the rodent variants are functional, as they lack one or

more transmembrane domains. Human GPR83 shares 89.5% sequence homology with mice, and 88% homology with rats, while mice and rats share 97% sequence homology.^{12,20–24} The mouse GPR83 shares the highest sequence identity with the tachykinin family of receptors, with ~31–33% shared identity with the subtypes.¹² The highest degree of identity is within the transmembrane domains, with 42.4% overall identity, while the lowest homology is observed in the N-terminus, C-terminus, and third cytoplasmic loop.^{12,22} However, they do share a number of potential N-linked glycosylation sites in the N-terminus, and several putative phosphorylation sites for protein kinase C and protein kinase A in the third intracellular loop and C-terminus.²²

1.2 Discovery of proSAAS and the Signaling Peptide PEN

A critical step in the identification of an endogenous ligand for GPR83 was the discovery of a family of peptides all derived from a precursor protein termed proSAAS, so named for the amino acid sequence of one of the peptides, SAASv (Ser-Ala-Ala-Ser).² ProSAAS is a ~260 amino acid precursor protein that is processed into at least five peptides, SAAS, GAV, PEN, bigLEN, and littleLEN (Fig. 1).^{2,10} The human form shares 84% homology with mouse/rat while the mouse and rat forms share 97% sequence homology.^{5,7}

The proSAAS peptide family was discovered by neuropeptidomic studies that characterized peptides in the brain of CPE^{fat/fat} mouse—a carboxy-peptidase E (CPE) mouse with a known mutation that renders the enzyme inactive.² Since CPE is an essential enzyme in the complete processing of peptide intermediates,²⁵ the lack of enzymatic activity in CPE^{fat/fat} results in an enrichment of these intermediate peptides, as compared to the control mice.^{25,26} Using mass spectrometry, researchers were able to screen for these enriched peptides in the CPE^{fat/fat} mouse brain, and thus identified the family of proSAAS peptides.²

Previous studies reported that mice lacking proSAAS exhibited decreased body weight,²⁷ whereas mice with the overexpression of pro-SAAS exhibited diabetes and obesity.²⁸ Given the potential importance of this novel peptide system, our group sought to identify the receptors through which they might be signaling. Because of the strong expression of proSAAS in hypothalamic tissue, we started to narrow down the list of potential receptors by cross checking orphan receptors that are expressed in the hypothalamus with those expressed in specific cell lines that also show strong binding and signaling of the proSAAS peptide.²⁹ After finding that bigLEN binds and activates the orphan receptor GPR171, we used a similar strategy to deorphanize GPR171 orphan receptor GPR83 as the receptor that binds PEN (see Section 4 for further details).³

1.3 ProSAAS Expression and Function

ProSAAS is highly expressed in numerous regions of the rodent brain, but it is especially enriched in the hypothalamus, hippocampus, amygdala and other limbic regions. Data from the Allen Brain Human Atlas suggests that this expression pattern is similar in human and rodents (Fig. 2). As a relatively new peptide family, there have been a limited number of studies looking at the role of these peptides in rodent behavior. However, preliminary studies suggest that proSAAS peptides are involved in numerous behaviors, including anxiety, as well as feeding and metabolism.^{9,27,28,30,31} Specifically, proSAAS knockout mice show

anxiety-like behavior in the open-field and elevated O-mazes.²⁷ Additionally, treatment with cocaine results in a significant down-regulation of proSAAS peptides in mouse nucleus accumbens (NAc) and ventral tegmental area, two brain regions highly related to reward. proSAAS knockouts also are less susceptible to cocaine and amphetamine-induced increases in locomotor activity and behavioral sensitization.³⁰ ProSAAS knockout mice show a slight decrease in body weight, while a global overexpression of proSAAS results in increases in body weight after 10 weeks of age.^{27,28} Finally, injection of PEN antibody into the mouse third ventricle results in up to a 50% decrease in food intake that persists up to 14 h.⁹

2. EXPRESSION AND SIGNIFICANCE OF GPR83 IN THE BRAIN

2.1 Expression of GPR83 in the Mouse Brain

Initial expression studies examining GPR83 in the mouse brain found high mRNA expression in numerous regions.⁴ Subsequently, several other studies characterized the region-specific expression of this receptor in mouse, rat and human.^{21,23,24}

In the mouse brain, Pesini et al.²³ noted the strongest expression of GPR83 in regions that regulate reward processing, fear responses and metabolic function. These results are in agreement with data from the Allen Brain Developing Mouse Brain Atlas (Fig. 3). Of note is the expression of GPR83 in the ventral striatum, including the nucleus accumbens and olfactory tubercle (Fig. 3A and E). While both the NAc and olfactory tubercle are involved in reward, the NAc is the central region of the brain where reward signals are processed. Dopamine, the neurotransmitter that is most important in reward signaling, is released from dopaminergic neurons originating in the ventral tegmental area. Dopamine then activates either D1-type or D2-type dopamine receptors expressed on medium spiny neurons, the principal neurons in the NAc. The remaining neurons are either GABAergic or cholinergic interneurons. The neurons that specifically express GPR83 are currently unknown.

GPR83 is also highly expressed in the basolateral amygdala (BLA) and subiculum, which both send dense glutamatergic projections to the NAc^{32,33} (Fig. 3C, D, I, and O). These projections are responsible for encoding reward valence³⁴ and regulating drug reinstatement.³⁵ In fact, direct activation of BLA to NAc projections is sufficient to induce reward seeking behaviors.³² The subiculum, the output region of the ventral hippocampus, sends contextual and spatial information to the NAc to produce drug-context associations.³⁶ Projections from the subiculum to the NAc are selectively potentiated in response to cocaine conditioning, mediate cocaine-induced locomotion and regulate cocaine seeking behavior.^{33,37,38} Expression of GPR83 in these brain regions suggests the receptor could function as a modulator of reward valence and learned associations between the reward and contextual cues.

GPR83 has ~fivefold higher expression in the lateral habenula (whose activation produces aversion) as compared to the expression in the medial habenula, whose activation produces positive reinforcement (“reward”).^{39,40} Given that the regulation of the reward system by the habenula is mediated by direct modulation of dopaminergic neuron activity, the differential expression of GPR83 in this brain region places the receptor in a position to directly regulate reward signals.

Processing of emotional memory and fear responses is another function of the amygdala. Incoming sensory information is received in the lateral amygdala, which sends projections to the BLA, a region of the amygdala that has dense expression of GPR83 (Fig. 3C and I). The BLA contains predominantly glutamatergic neurons, in addition to parvalbumin and somatostatin expressing GABAergic interneurons.^{41,42} The central nucleus of the amygdala (CeA), which receives inputs from the BLA, also has dense expression of GPR83 (Fig. 2C and J). In contrast to the BLA, the CeA contains GABAergic MSNs, similar to the striatum, some of which express somatostatin.^{41,42} Activation of specific neuronal subtypes that form the circuit between the BLA-CeA can either produce a fear or an anxiolytic response.⁴² An essential key to understanding the role of GPR83 in these processes is to characterize the neuronal subtypes in which GPR83 is expressed.

In the hypothalamus, GPR83 is expressed in the arcuate nucleus and the preoptic area^{43–45} (Fig. 3B, C, F, and H). GPR83 is localized to agouti related peptide (AgRP)- and ghrelin receptor (Ghr) expressing neurons. AgRP neurons, which co-release NPY and GABA, sense peripheral hormonal signals such as insulin, leptin and ghrelin which act to increase feeding behaviors.^{46–49} In contrast, activation of proopiomelanocortin (POMC) neurons, also localized in the arcuate nucleus, decreases feeding behaviors. In fact, studies demonstrate that GABA release from AgRP neurons increases inhibitory currents on POMC neurons and regulates energy expenditure.⁵⁰ The localization of GPR83 in this brain region implies a role for this receptor in modulating feeding behavior. In addition to its expression in the arcuate nucleus, GPR83 is expressed in warm-sensitive neurons, which are neurons that increase their firing in response to small (1–3 °C) changes in local temperature, of the preoptic area that are involved in regulating core body temperature and fever responses (Fig. 3B and F).⁴⁴ GPR83 expression in AgRP and warm-sensitive neurons suggests a significant role in regulating metabolism, energy expenditure and regulation of core body temperature, which will be discussed later in this review.^{43,45}

The expression of GPR83 in the hippocampus and mammillary bodies suggests a role in regulating memory-related behaviors (Fig. 3C, D, K–M, and P). The hippocampus encodes contextual and spatial memories via the excitatory trisynaptic pathway. Here incoming information enters the hippocampus via projections from the entorhinal cortex, which synapses onto the dendrites of granule cells in the dentate gyrus. These neurons send projections to the dendrites of CA3 pyramidal neurons, which then synapse onto the dendrites of CA1 pyramidal neurons. The cellular basis of learning and memory is thought to be encoded at the synapse between CA3 and CA1 pyramidal neurons. In fact, in the mouse, GPR83 expression is strongest in the CA3¹ and dentate gyrus (our observations), suggesting that GPR83 could be involved in regulating the encoding of memories.

Another indication that GPR83 may play a role in the regulation of spatial and contextual memories is based on its high expression levels in the mammillary bodies (Fig. 3D and P).²³ One main output from the hippocampus is through the subiculum, as mentioned previously. The mammillary nuclei are a relay between subiculum outputs from the hippocampus to thalamic nuclei and have also been implicated in memory formation.⁵¹ There is some evidence to suggest that GPR83 does in fact modulate spatial memories.⁵²

2.2 Differential GPR83 Expression Between Mouse, Rat and Human

As compared to mouse GPR83, rat and human share 99% and 89.5% sequence identities respectively,^{20,24,53} suggesting that the function of GPR83 is similar across species as well. The expression pattern of GPR83 among mouse, rat, and human are generally similar with some differences in the levels of expression (Fig. 4).^{23,24} One difference is that GPR83 expression in the mouse hippocampus is highest in the CA3 region, while in rat, expression was reported to be equal throughout all regions of CA areas and dentate gyrus.^{22,24} In contrast, in human hippocampus, GPR83 expression is strong in the dentate gyrus and CA3 regions, similar to the findings in mice.²¹ Another difference between mouse and rat is GPR83 expression in the striatum. In mouse brain, GPR83 is found throughout the striatum, including core and shell regions of the nucleus accumbens with scattered expression throughout the caudate putamen.^{22,23} In rat brain, GPR83 expression in the striatum is lower with conflicting reports on whether expression is localized to the core or shell regions of the nucleus accumbens.^{22,24} Data from human suggests that while GPR83 is expressed in this region it is at a moderate level (Fig. 4). Overall in the human, GPR83 expression is strongest in hypothalamic, hippocampal and amygdaloid regions with moderate expression in striatal regions (Fig. 4).

One striking difference is that GPR83 is expressed in the cerebellum in the rat but not the mouse. Moreover, in the human cerebellum the strongest expression of GPR83 is in the cerebellar granular cell layer,²¹ suggesting that GPR83 could play a role in regulating motor learning and coordination in humans. Overall, the pattern of expression of GPR83 suggests that the receptor plays a role in emotion, learning, reward processing and metabolic functions.

2.3 Regulation of GPR83 Expression in the Brain

The regulation of GPR83 expression in specific brain regions may be altered by neuroadaptations that occur due to stress, reward pathway activation, neuroendocrine responses and learning and memory. The first reports on GPR83 identified that the glucocorticoid agonist dexamethasone regulates the expression of the receptor in vitro.⁴ Since glucocorticoids are released and bind to glucocorticoid receptors in response to activation of the hypothalamic–pituitary–adrenal (HPA) axis, this implies that GPR83 plays a role in stress responses. In addition, in vivo studies demonstrated that oral dexamethasone treatment produces a decrease in GPR83 expression in a variety of brain regions, including striatum, hippocampus and hypothalamus.^{54,55} Similarly, both diet-induced obesity and fasting produces decreases in GPR83 expression in the hypothalamus,⁴³ suggesting that GPR83 expression is compensating for changes in metabolic function. In terms of drug reward, it has been shown that behavioral sensitization to amphetamine increases GPR83 expression in the prefrontal cortex for up to 7 days after withdrawal.²² A recent study demonstrated that GPR83 may play a role at the intersection of reward and learning as it was demonstrated that GPR83 expression is up-regulated in cultured hippocampal astrocytes following exposure to dopamine.⁵⁶ Finally, depletion of the long chain fatty acid docosahexaenoic acid, which results in a decrease in the rate of learning of an olfactory discrimination task, decreases GPR83 expression in the olfactory bulb.⁵⁷ These studies begin

to highlight the variety of neurological functions wherein GPR83 may be playing a critical role, and which need to be explored further using mouse physiology and behavior.

2.4 Role of GPR83 in Hypothalamic Function

The first reports of the in vivo impact of centrally expressed GPR83 demonstrates an important role for GPR83 in metabolic function. A shRNA viral mediated knockdown of GPR83 in the preoptic area of the anterior hypothalamus (POA) decreased core body temperature and increased body weight in mice with no changes in food intake.⁴⁵ As mentioned earlier, GPR83 is expressed in warm-sensitive neurons in the POA (Fig. 3B and F), which are responsible for maintaining core body temperature and fever responses. These neurons are GABAergic since they express mRNA for GAD 1, a GABA synthesizing enzyme, in addition to somatostatin and dynorphin peptides among others.⁴⁴ Warm-sensitive neurons of the POA project to the rostral raphe pallidus. The POA-rostral raphe projection is involved in fever initiation since immune signals that block the POA mediated inhibition of the rostral raphe result in a rise in body temperature.⁵⁸

Further support for the role of GPR83 in hypothalamic function was demonstrated using GPR83 transgenic mice.⁴³ Under normal conditions GPR83 KO mice did not exhibit changes in body weight, food intake, glucose tolerance or insulin sensitivity. However, these mice exhibited a decrease in fat mass at 18 weeks of age.⁴³ A significant role for GPR83 in metabolic function was uncovered when GPR83 WT and KO mice were treated with ghrelin. Peripheral ghrelin treatment increases food intake in wild type mice, an effect that is exacerbated in GPR83 KO mice. Additionally, chronic central treatment with ghrelin produces significant enhancement of body weight, food intake and fat mass in GPR83 KO mice compared to WT mice. Moreover, GPR83 KO mice had increases in plasma leptin and decreases in free fatty acid levels. Given that GPR83 expression in the arcuate nucleus of the hypothalamus is localized to neurons that co-express the ghrelin receptor, these data strongly support the notion that GPR83 is influencing ghrelin receptor activity to regulate feeding behavior.

Interestingly, a study examining the effect of a high fat diet in GPR83 WT and KO mice found that the loss of GPR83 protected mice from diet-induced obesity, since they exhibited decreased weight gain and fat mass and an improved glucose tolerance compared to WT controls on the same high fat diet.⁴³ Moreover, although the GPR83 KO mice increased food intake compared to WTs, they did not gain as much weight as WTs on the same diet. This effect was due to increases in energy expenditure in GPR83 KO mice that were not associated with increases in locomotor activity. Increases in AgRP, NPY, Hcrt and Ghsl1a expression in the hypothalamus of GPR83 KO mice led the authors to suggest that GPR83 interacts with additional signaling mechanisms to regulate energy metabolism.⁴³

2.5 Role of GPR83 in Stress, Reward and Learning and Memory

The expression of GPR83 in the amygdala, nucleus accumbens, prefrontal cortex and hippocampus (Figs. 3 and 4) suggests that this receptor plays a role in stress responses, the reward pathway and learning and memory. This is supported by studies by Vollmer et al.⁵² using GPR83 KO mice. Using an acute restraint stress model, the authors demonstrated that

GPR83 KO mice are protected against stress-induced anxiety, as compared to WT mice. However, GPR83 KO mice did not show any differences in serum corticosterone levels in response to restraint stress, suggesting that the mechanism of resilience is not directly related to the activation of the HPA axis. One possibility is that cell type expression of GPR83 in a brain region such as the amygdala is producing these anxiolytic effects.

Previous studies reported that repeated treatment with amphetamine to produce behavioral sensitization increases GPR83 expression in the prefrontal cortex.²² This suggests that GPR83 expression is involved in the neuroadaptations that occur in response to drugs of abuse, which result in drug dependence. To investigate GPR83's role in the reward system, GPR83 WT and KO mice were tested in a two-bottle choice sucrose drinking paradigm where mice are presented with one bottle containing water and the other containing increasing percentages of sucrose.⁵² This test is used to measure anhedonia (inability to feel pleasure), which can be induced by chronic stressors that result in depression. Neuroadaptations that result in anhedonia typically occur in the reward system of drug dependent individuals. In this assay, GPR83 KO mice had an increase preference for lower doses of 1% sucrose and not 3%, 10% or 30% sucrose.⁵² Perhaps the lack of sucrose preference at higher doses could be due to a ceiling effect of the ability of sucrose to activate the reward pathway. Also, sucrose is a natural reward and it is not known whether natural reward increase GPR83 expression in a similar way as they do drugs of abuse, such as amphetamine. Overall, these studies indicate that GPR83 does play a role in reward.

The pattern of GPR83 expression in the hippocampus, prefrontal cortex and amygdala suggests that this receptor may regulate spatial and fear learning (Fig. 3).^{21,23,24} Spatial learning was assessed in GPR83 WT and KO mice using the Morris water maze where mice use spatial cues to learn the location of a platform hidden beneath the surface of the water.⁵² In this test, GPR83 KO mice displayed a borderline delay in the acquisition of this task and no differences in recall 24 h following training compared to WT mice. Since the Morris water maze is a hippocampal-dependent task, this suggests that GPR83 is playing a minor role in hippocampal function.⁵⁹ Contextual fear conditioning was used to determine the role of GPR83 in processing fearful stimuli. This task involves circuitry between the amygdala and prefrontal cortex and hippocampus. In GPR83 KO mice there were no differences in the acquisition, recall or extinction of fear conditioned cues suggesting that GPR83 does not play a role in these processes.⁵²

One caveat of these studies is that they primarily utilized the global GPR83 KO mice instead of a region-specific knock down. In studies where the global GPR83 KO produces a biological effect in the animal, it is still unclear precisely which brain region may be responsible for producing the effect. An example is the case in which GPR83 KO alleviates anxiety produced by acute restraint stress. Given that GPR83 is highly expressed in two different subregions of the amygdala, the basolateral and central nucleus of the amygdala, as well as the bed nucleus of the stria terminalis, all of which are known to regulate anxiety and stress responses, it is not clear which region is responsible for these behavioral phenotypes. In examples where there was no effect or mild effects on spatial and fear learning with global KO of GPR83, one cannot rule out the potential for compensatory mechanisms, which may mask GPR83's role.

To date only one study has explored GPR83's direct regional effects by region specific knock-down of GPR83 in POA,⁴⁵; a region known to regulate core body temperature and fever responses. Thus, additional studies are needed to evaluate the role of GPR83 in specific brain regions. A critical step toward completing this goal was achieved by deorphanization of GPR83.³ By understanding the signaling pathways activated by GPR83 binding to its endogenous ligand, we can begin to explore how these signaling mechanisms affect neuronal function to produce behavioral effects.

3. ROLE OF GPR83 IN IMMUNE FUNCTION

3.1 Expression of GPR83 in Immune Cells

Although GPR83 was originally cloned in a murine thymoma cell line,⁴ little was known regarding its expression on specific immune cells or its role in immune function. In 2006, Sugimoto and colleagues identified GPR83 as a marker for a specific pool of naturally occurring, activated T-regulatory cells, CD4⁺ CD25⁺ Foxp3⁺ T_{regs}, which are critically important for immunological self-tolerance.¹⁴ Deletion of either CD25 (also known as IL-2 receptor alpha chain) or the transcription factor Foxp3 (Forkhead box P3) results in a loss of natural T_{regs}, likely through the inability of naïve CD4⁺ CD25⁻ T_{regs} to mature, ultimately resulting in severe autoimmune disease.^{60–63} Interestingly, GPR83 and Foxp3 show a very strong positive correlation in expression in the natural T_{reg} population in rodents and humans, and both are now commonly used as markers for this population of activated cells.

A second population of T-regulatory cells, sometimes referred to as suppressor cells, or T_{supp}, have also been shown to express GPR83. These cells, which are CD8⁺ CD25⁺ Foxp3⁺, have a similar suppressor function as the CD4⁺ T_{regs}, but scientists have a limited understanding of their precise biological role.^{16,64} T_{supp} cells have been implicated in inflammatory diseases, specifically in human inflammatory bowel disease and asthma.⁶⁴ As with CD4⁺ T_{regs}, following activation, CD8⁺ T_{supp} up regulate both FoxP3 and GPR83.¹⁶

No direct studies have been published looking at the protein expression of GPR83 in other immune cell populations, such as B cells and NK cells. However, according to various online databases (ARCHS and BioGPS), which compile data from various RNA-seq and microarray studies, GPR83 is also expressed in a variety of immune cells including B cells, granulocytes, and neutrophils.^{65,66}

3.2 Significance of GPR83 in Immune Function

Though the expression of GPR83 on natural CD25⁺ Foxp3⁺ T_{regs} is well established, the functional significance of this expression is still controversial. Transfection of Foxp3 leads to an increase in GPR83, and GPR83 expression is increased in mature CD4⁺ CD25⁺ T_{regs} that also express Foxp3, suggesting a close association between GPR83 and these mature T_{regs} (Fig. 5).^{13,14,19} Hansen and colleagues showed that under inflammatory conditions in vivo, GPR83 overexpression in naïve CD4⁺ CD25⁻ T cells is able to induce peripheral maturation of these cells, as indicated by an increase Foxp3 expression, and confer anti-inflammatory properties.¹³ However, it is important to note that when GPR83 is over-expressed on the same cells in vitro, there is no increase in Foxp3, nor are the cells able to

suppress proliferation of naïve CD4⁺ CD25⁻ T cells. Furthermore, in vivo expression of GPR83 transfected naïve CD4⁺ CD25⁻ T cells is not able to induce peripheral maturation under normal or non-inflammatory conditions.^{13,19} This suggests that the ligand or mode for activation of GPR83 leading to maturation of naïve T_{regs}, directly or indirectly, is only available under inflammatory conditions in vivo.

Lu et al. created a GPR83 knockout mouse to seek a better understanding of the role of the receptor in CD4⁺ CD25⁻ T cell maturation.¹⁵ They found that the lack of GPR83 did not hinder T_{reg} development, nor did it alter the suppressive abilities of mature T_{regs}.¹⁵ Additionally, Toms et al.⁶⁷ also generated a GPR83 knockout mouse, and they did not observe any differences in expression levels for naïve or mature T_{regs} in numerous different tissues, including spleen, lymph nodes, thymus, and the lamina propria of the colon.⁶⁷ In their model of colitis, lack of GPR83 did not affect the acquisition or suppression of the inflammatory disease.⁶⁷

Taken together, no one consensus has been reached regarding the precise role of GPR83 in immune function. However, the immune landscape is quite complicated, and there are likely numerous factors and systems at play that have not been taken into account. Each study utilized different systems (i.e., skin sensitization vs intestinal inflammation) and under different conditions (i.e., overexpression of GPR83 vs depletion of GPR83), making direct comparisons and conclusions difficult to deduce. Furthermore, due to the complicated nature of immune activation, maturation, and function, there is likely significant overlap that essentially results in redundancies that are able to manage loss of function in one system. While the lack of GPR83 appears to be dispensable for proper immune function, overexpression of the receptor does suggest the ability to play a role in anti-inflammatory processes. From a therapeutic point of view, perhaps targeting a system with more of a selective, modulatory role is actually desirable, as the chance for off-target effects or undesirable side effects may be avoided. Future research into GPR83 in immune function must take these factors into consideration, and researchers may even consider a more integrated, systems-biology approach to these multi-faceted issues.

In summary, at this point, there are few definitive roles that can be attributed to GPR83 in terms of immune function. However, we do know that its expression is tightly correlated with that of Foxp3 and that its role in producing functional natural T_{regs} requires an inflammatory in vivo environment.^{13,19} While it may not be a critical component of T_{reg} maturation or function, or a primary target for immune function, it may be a viable secondary target for modulatory regulation of immune responses.

4. CURRENT UNDERSTANDING OF GPR83 AND PEN

4.1 The Deorphanization of GPR83

From previous studies showing that intracerebroventricular injection of antibodies to the proSAAS derived peptides bigLEN or PEN leads to a reduction in acute feeding,⁹ the receptor for these two peptides were predicted to localize in feeding centers in the hypothalamus. Hence orphan receptors enriched in the hypothalamus were screened for activation by PEN. From these studies it was found that the PEN peptide specifically

activated G-protein signaling via GPR83, a receptor that was known to be expressed in the AgRP neurons that regulate feeding behavior.^{3,43} Binding studies demonstrated that PEN specifically binds to brain regions that also express GPR83, including the striatum, olfactory bulb, hypothalamus and hippocampus. Moreover, these studies identified that stimulation of GPR83 by PEN activates $G\alpha_i$ mediated signaling in the hippocampus and $G\alpha_q$ signaling in the hypothalamus, indicating that GPR83 may differentially regulate downstream pathways depending on the brain region in which it is expressed. Previous studies had suggested that the neuropeptide NPY may be a ligand for GPR83 because of its high homology of GPR83 with the NPY- Y_2 receptor.⁶⁸ However Gomes et al.³ demonstrated that NPY was not able to displace PEN at physiological concentrations. Together, these studies identified that PEN is the endogenous ligand for GPR83.

5. CONCLUSIONS

5.1 Relationship Between GPR83, Stress, Reward, and Immune Function: Future Research Considerations

GPR83 has a very interesting expression pattern, as it is highly expressed in the central nervous system (CNS) and immune systems of rodents and humans.^{13,20–24,53} For decades it was assumed that these two biological systems functioned independently and that neither was able to affect or influence the other. More recently, scientists have found that not only does the immune system greatly affect the CNS but that the CNS is able to influence the peripheral immune system.^{54,69–82} Some of the first indications of such interactions were in the context of stress, specifically following the activation of the HPA-axis, which results in release of corticosterone in mice (cortisol in humans).^{83–87} Generally speaking, acute stress and subsequent release of corticosteroids results in temporary pro-immune or immuno-protective responses, while chronic stress can depress immune function and result in aberrant immune-pathological states.^{83,85,88,89} Furthermore, chronic exposure to corticosteroids results in numerous cellular changes in the brain, and chronic stress is a strong risk factor for the development of anxiety, depression, addiction, and other psychological disorders.^{80,83,86,90,91} GPR83 was discovered as being activated by the synthetic glucocorticoid dexamethasone, suggesting that GPR83 may be involved in the stress response, and indeed, knocking out GPR83 in mice leads to a stress protective phenotype.⁵² Further research needs to be conducted regarding the role of the immune system in response to stress and the potential protective effects GPR83 may play centrally or peripherally. Because its neuro-immuno-endocrine expression pattern, it is likely that there is a strong interaction between GPR83, stress, and immune function that should be further explored.

5.2 The GPR83-PEN Neuropeptide System as a Novel Therapeutic Drug Target

Despite a somewhat limited understanding of the biological role of GPR83, development of small molecule compounds that can bind to this receptor will assist in the functional characterization of the receptor in a variety of psychological and immune disease states. For example, a peripherally restricted compound could target GPR83 function in only immune cells. As discussed above, knockout of GPR83 does not appear to cause gross immune deficiencies. However, GPR83 expression does influence immune response to certain

inflammatory conditions in vivo, suggesting that it would be an excellent drug target to modulate immune function in autoimmune or inflammatory conditions.

In the context of the CNS alone, GPR83 knockout mice are resistant to stress-induced anxiety.⁵² GPR83 compounds may be viable novel targets for the development of anxiolytics with fewer side effects than currently used compounds such as benzodiazepines and SSRI/SNRIs, which can cause drowsiness, interact with other drugs, or be abused. Furthermore, GPR83 antagonists may be useful as prophylactics in the prevention of stress-induced anxiety for people in high-stress environments that are at a higher risk for development of PTSD and other stress-related psychological issues.

GPR83 knockout mice also show resistance to diet-induced increases in obesity and diet-induced glucose intolerance, and sequestration of PEN in the hypothalamus using antibodies significantly reduces feeding, suggesting an important role for GPR83 in feeding and metabolism.^{9,43} Therefore, GPR83 may also be a novel target for those struggling with eating disorders and obesity, or even diet-induced diabetes.

To date there has been one study identifying GPR83 as a potential factor in the development of obstructive sleep apnea in human patients.⁹² Here the authors found the genetic loci, which contain the GPR83 gene, as a risk factor for this disorder. These studies identify that GPR83 is expressed in brain regions related to this disorder and that GPR83's expression on T_{regs}, in the immune system, also may be related to the severity of obstructive sleep apnea. Together these are the first indication that GPR83 is playing a role in the intersection of the brain and immune system.

5.3 Summary

In summary, GPR83 and its endogenous ligand, PEN, exhibit region specific expression in neuroendocrine tissues, and the receptor is highly expressed in various cells of the immune system. It has been implicated in numerous behaviors, including stress, anxiety, reward, as well as feeding and metabolism. While much more research is required to fully elucidate the role of GPR83 and PEN in all of these biological systems, this neuropeptide-receptor system has a strong potential as a novel target for future drug development. Certainly, from both a basic biology perspective, as well as for therapeutic development, compounds targeting this receptor will open a wide door for future research.

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Fig. 1.

Proteolytic processing of proSAAS into known signaling peptides. The precursor protein and subsequent processed peptides are indicated by color. The top row indicates the amino acid cleavage sites. The indicated residues are removed by carboxypeptidase (CPE or CPD). Below the precursor protein, the arrows indicate sites that are cleaved by furin/CPD (dashed arrow indicates potential but less efficient cleavage from furin). The box indicates enzymatic activity that occurs within the secretory vesicles. Here bigSAAS is cleaved into KEP and littleSAAS, the middle portion into GAV, and the final portion is cleaved into PEN and bigLEN, which is further processed into littleLEN and LLPP. Primary conversion of these peptides is via PC1/3 and CPE. *From Wardman JH, Fricker LD. ProSAAS-derived peptides are differentially processed and sorted in mouse brain and AtT-20 cells. PLoS One. 2014;9:e104232, open source CCL.*

Fig. 2.

The Allen Human Brain Atlas microarray data demonstrating differential expression of proSAAS across several brain regions. Expression data for six adults were obtained using two probes for PCSKIN (proSAAS). Data for each probe was averaged within each patient and then the average across patients was plotted. Data are represented as normalized z-scores and plotted from highest expression to lowest. Technical information about microarray setup and analysis are available at www.alleninstitute.org. The entire data set is available at <http://human.brain-map.org>.

Fig. 3. GPR83 mRNA expression from the Allen Brain Developing Mouse Atlas. Coronal mouse brain sections were probed with GPR83 in situ hybridization probes. Images were obtained at <http://mouse.brain-map.org/experiment/show?id=72338696>. (A–D) Images from whole mouse brains probed for GPR83. Specific sub regions are outlined in black and representative larger images are displayed in (E–P). Nac, nucleus accumbens; POA, preoptic area; BNST, bed nucleus of the stria terminalis; Arc, arcuate nucleus; BLA, basolateral amygdala; CeA, central nucleus of the amygdala; CA1, Cornu Ammonis 1 (hippocampus); CA3, Cornu Ammonis 3 (hippocampus); DG, dentate gyrus; Ent Ctx, entorhinal cortex; Sub, subiculum and MN, mammillary nuclei. *Image Credit: Allen Institute.*

Fig. 4.

GPR83 expression in the human brain in coronal 20-mm-thick sections of hemisphere showing distribution of GPR83 (JP05) mRNA using antisense riboprobe. AB, accessory basal amygdala nucleus; Amy, amygdala; Arc, arcuate; B, basal amygdaloid nucleus; BNST, bed nucleus stria terminalis; Ce, central amygdala; CN, caudate nucleus; Cop, cortical amygdaloid nucleus, posterior; Cl, claustrum; EC, entorhinal cortex; End, endopiriform cortex; f, fornix; GPe, external segment of the globus pallidus; L, lateral amygdaloid nucleus; Me, medial amygdala; ot, optic tract; PAC, periamygdaloid cortex; Pu, putamen;

PVN, paraventricular hypothalamic nucleus; SLEA, sublenicula extended amygdala; VMH, ventromedial hypothalamic nucleus. *Adapted fig. 2B from Brezillion S, et al. Distribution of an orphan G-protein coupled receptor (JP05) mRNA in the human brain. Brain Res. 2001; 921(1-2):21-30, by permission of Elsevier.*

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Fig. 5.

Co-expression of GPR83 and Foxp3 in regulatory T-cells. (A) RT-PCR quantitative analysis of GPR83 in fresh or activated spleen and lymph node cells (left), as well as CD25⁻ CD4⁺ T_{regs} cells spleen and lymph node cells transduced with Foxp3- or mock- transduced (right). (B) Spleen and lymph cells were purified and freshly prepared or activated for 3 days, followed by staining with GPR83 antibodies (green) and Foxp3 antibodies (red). In fresh cells (top panel), GPR83 and FoxP3 are co-expressed in a few cells; however, following activation (bottom panel), both GPR83 and FoxP3 are greatly up-regulated in the same cell populations. *Modified figs. 4A and 5B from Sugimoto N, et al. Foxp3-dependent and -independent molecules specific for CD25 + CD4 + natural regulatory T cells revealed by DNA microarray analysis. Int Immunol. 2006; 18(8):1197–1209, by permission of Oxford University Press.*