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ANTI-INFLAMMATORY NEUROPEPTIDES: A NEW CLASS OF ENDOGENOUS IMMUNOREGULATORY AGENTS

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Abstract

Resolution of inflammation and induction of immune tolerance are essential to stabilize immune homeostasis and to limit the occurrence of exacerbated inflammatory and autoimmune conditions. Multiple mechanisms act together to ensure the re-establishment of immune homeostasis and maintenance of tolerance. The identification of endogenous factors that regulate these processes is crucial for the development of new therapies for inflammatory/autoimmune conditions. Neuropeptides produced during an ongoing inflammatory response emerged as endogenous anti-inflammatory agents that participate in processes leading to the resolution of inflammation and maintenance of tolerance. Anti-inflammatory neuropeptides and hormones such as vasoactive intestinal peptide, urocortin, adrenomedullin, melanocyte stimulating hormone, ghrelin and cortistatin have beneficial effects in a variety of experimental inflammatory and autoimmune models. Their therapeutic effect has been attributed to their capacity to downregulate innate immunity, to inhibit antigen-specific T_H1-driven responses, and to generate regulatory T cells. Finally, some of these neuropeptides have been identified as mediators of innate defense acting as natural antimicrobial peptides. Here we present the research findings in the neuropeptide immunoregulatory field, and examine possible therapies based on anti-inflammatory neuropeptides and hormones as a new pharmacologic platform.

Keywords

Inflammation; Autoimmunity; Regulatory T cells; Antimicrobial peptides; Tolerance; Neuropeptides; Hormones

1. Introduction

Elimination of pathogens through the activation of the immune system followed by reestablishment of immune homeostasis requires multiple interactions between various types of immune cells and between the immune and neuroendocrine systems. Among neuroendocrine

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mediators, neuropeptides, expressed and released primarily but not exclusively by the nervous system, have profound effects on the immune response.

Recent developments in the field of immunoregulatory neuropeptides led to the special named series “Neuropeptide Regulation of Immunity” published in *Brain Behavior and Immunity*. The studies published in this series encompass the anatomical localization of peptidergic nerve fibers in close proximity to lymphocytes (Shibata et al, 2008), the potent anti-inflammatory activity of neuropeptides/hormones such as vasoactive intestinal peptide (VIP), endomorphin 1 and 2, and alpha-melanocyte stimulating hormone (α -MSH) (Gonzalez-Rey et al, 2008; Gutierrez-Canas et al, 2008; Taylor and Kitaichi, 2008; Colombo et al, 2008; Anton et al, 2008), the regulation of neuropeptide receptor expression in immune cells (Murthy et al, 2008; Vornhof-DeKrey et al, 2008a and b), and the dependence of immune parameters such as thymus involution and T lymphocyte output, in addition to growth hormone expression, on signalling through ghrelin receptors (Yang et al., 2008).

The review concluding this special named series reports on various mechanisms involved in the activity of a growing number of anti-inflammatory neuropeptides and discusses their possible use as new therapeutic agents.

Following the elimination of pathogens, inflammation has to be resolved to re-establish homeostasis. The consequences of uncontrolled activation of innate and adaptive immunity and of sustained production of inflammatory mediators are deleterious for the host. Acute inflammation can lead to chronic inflammation, scarring and tissue destruction, and eventually to organ failure (Goodnow, 2007).

Although there is substantial information on the molecular basis of the initiation of an inflammatory process, we know much less about the mechanisms that resolve inflammation. Recently, it has been recognized that inflammatory responses are self-controlled by endogenous anti-inflammatory mediators secreted by the host innate immune system during an ongoing inflammatory process, and that the ability to control an inflammatory state depends on the local balance between pro- and anti-inflammatory factors (Nathan, 2002). In healthy individuals, the immune response against self antigens is prevented and controlled by long-term tolerance, established and controlled by central thymic clonal deletion of self-reactive T cells, induction of anergy in self-reactive T cells in the periphery and generation of antigen-specific regulatory T cells (Treg) which suppress the activity of self-reactive effector T cells (Bluestone, 2005).

A number of traditional immunosuppressive cytokines, such as IL-10, IL-13 and TGF- β 1, have been shown to also play an important role in tolerance, particularly in the generation of Treg (Wan and Flavell, 2006). We and others established that certain classical neuroendocrine mediators, i.e. neuropeptides and hormones also play an essential role in resolving inflammation and maintaining tolerance through the induction of antigen-specific Treg (Gonzalez-Rey et al., 2007a).

Here we review recent developments regarding the effects of anti-inflammatory neuropeptides on immune tolerance and resolution of inflammation, and highlight the effectiveness of using neuropeptides in treating several inflammatory and autoimmune disorders.

2. Neuropeptides as biochemical mediators in neuro-immune crosstalk

In the last three decades, it became evident that the bi-directional connection between the neuroendocrine and immune systems serve to mount a variety of coordinated responses to external/internal danger. The immune system signals the brain to respond to pathogen infection and inflammation, leading to the febrile response and profound effects on behavior (Sternberg,

2006). Conversely, the immune system is controlled by the CNS, mainly in response to environmental stress. This intimate network is based on a mutual biochemical language, that involves shared ligands (neuropeptides, hormones, cytokines) and the respective receptors. Glucocorticoids and norepinephrine are the classical examples of endogenous immunoregulatory agents produced by the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system, respectively, in response to stress or systemic inflammation (Sternberg, 2006). Moreover, various neuropeptides are released at the peripheral peptidergic endings of sensory and efferent nerves in close proximity to immune cells in response to various invasive and inflammatory stimuli (Shibata et al., 2008). Perhaps one of the most exciting findings was the recent discovery that some neuropeptides are also produced by immune cells, including neutrophils, lymphocytes, macrophages and mast cells (Gonzalez-Rey et al., 2007a).

Based on this crosstalk, it is reasonable to assume that peptides with neuroendocrine signaling functions produced by immune cells could contribute, in a cytokine-like manner, to the regulation of the immune response. From the growing list of neuropeptides currently thought to possess immunomodulatory actions (approximately 50), some neuropeptides have lately emerged as potential candidates for the treatment of inflammatory and autoimmune disorders. Vasoactive intestinal peptide (VIP), α -melanocyte stimulating hormone (α MSH), urocortin, adrenomedullin, cortistatin and ghrelin are neuropeptides that belong to different families but share certain immune regulatory characteristics (Table 1). First, they are produced by immune cells during inflammatory conditions or following antigenic stimulation. Second, they exert their effect through various G-protein-coupled receptors (GPCRs) expressed in various immune cells. Third, they signal mainly through the activation of cAMP/protein kinase A (PKA) and downregulate the activation of several transduction pathways and transcription factors essential for the expression of most of the inflammatory cytokines, chemokines and costimulatory factors, including the NF κ B, mitogen-activated protein kinases (MAPK), the interferon regulatory factor 1 (IRF1) and the activator protein 1 (AP1) (Chorny et al., 2006; Gonzalez-Rey and Delgado, 2007a; Yoon et al., 2003).

3. Neuropeptides counterbalance the inflammatory response

Numerous in vitro and in vivo studies demonstrated that VIP, α MSH, urocortin, adrenomedullin, ghrelin and cortistatin exhibit potent anti-inflammatory activities. These neuropeptides switch off the inflammatory response by regulating different critical levels of innate immunity (Chorny and Delgado, 2008; Chorny et al., 2008; Delgado et al., 2008a; Gonzalez-Rey et al., 2007a; Miksa et al., 2007; Wang et al., 2007): 1) they inhibit phagocytic activity, free radical production, adherence and migration of macrophages; 2) they reduce production of inflammatory cytokines (TNF α , IL-12, IL-6 and IL-1 β) and various chemokines by activated macrophages and microglia; 3) they downregulate expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2) and the subsequent release of nitric oxide and prostaglandin E2 by macrophages, DCs and microglia; 4) they stimulate production of anti-inflammatory cytokines such as IL-10 and IL-1Ra; 5) they decrease co-stimulatory activity of antigen-presenting cells (APCs) for antigen-specific T cells by downregulating expression of co-stimulatory molecules; 6) they inhibit secretion of late inflammatory mediator high-mobility group box 1 (HMGB1); 7) they reduce degranulation of mast cells; 8) they induce apoptosis in macrophages; and 8) they stimulate the peroxisome proliferator-activated receptor- γ (PPAR γ), an anti-inflammatory-related transcription factor.

4. Neuropeptides impair Th1 responses

A large body of literature has reported on the capacity of these neuropeptides to also regulate adaptive immunity. There is evidence that the presence of these neuropeptides can impair

activation/differentiation of Th1 cells, and in some cases (i.e., VIP) promote Th2-type responses (Delgado et al., 2004; Gonzalez-Rey et al., 2007a; Gutierrez-Canas et al., 2008). The mechanisms involved in regulation of the T_H1/T_H2 balance are not fully elucidated, with most of the current data provided by VIP studies. Various non-exclusive mechanisms have been proposed involving both direct actions on differentiating T cells and indirect regulation of APC functions (Delgado et al., 2002; 2004; Gonzalez-Rey et al., 2007a; Sharma et al., 2006; Voice et al., 2004). First, VIP inhibits production of T_H1-associated cytokine IL-12. Second, VIP induces CD86 expression in resting murine DCs, which is important for the development of T_H2 cells. Third, VIP has been shown to promote specific T_H2-cell recruitment by inhibiting CXC-chemokine ligand 10 (CXCL10) production and inducing CC-chemokine ligand 22 (CCL22) production, two chemokines that are involved in the homing of T_H1 cells and T_H2 cells, respectively. Fourth, VIP inhibits CD95 (FasL)- and granzyme B-mediated apoptosis of mouse T_H2 but not of T_H1 effector cells. Finally, VIP induces the T_H2 master transcription factors c-MAF, GATA-3 and JunB in differentiating murine CD4⁺ T cells, and inhibits T-bet, which is required for T_H1 cell differentiation.

An important point that is still unresolved is the action of neuropeptides on T_H17 responses. Because T_H17 cells are essential for the initiation and perpetuation of autoimmune responses, the effect of neuropeptides on T_H17 differentiation/activation needs urgent investigation. Different *in vivo* studies show that VIP administration reduces the presence of T_H17 cells in inflamed tissues and draining lymph nodes (Delgado et al., 2008b, Gonzalez-Rey et al., 2006a, 2006b, 2007b). However, because these neuropeptides significantly downregulate the production of a plethora of chemokines, it is not clear whether this effect is a consequence of reduced infiltration of immune cells, or a direct effect of Th17 differentiation and/or migration. Moreover, a recent study reported an unexpected finding, *i.e.* addition of VIP to TCR-activated CD4 T-cell cultures induced the generation of IL-17-secreting T cells (Yadav et al., 2008). Interestingly, the VIP-induced T_H17 cells only occurred in the presence of TGFβ1, and the differentiated cells showed a distinctive cytokine profile characterized by production of IL-17 and IL-22, but not IL-6 or IL-21 (Yadav et al., 2008). Unfortunately, this study does not elucidate whether the VIP-induced IL-17-secreting T cells are pathogenic or whether they have the capacity to transfer autoimmunity.

5. Neuropeptides induce regulatory T cells

VIP and αMSH have been reported to generate DCs with a tolerogenic phenotype, characterized by their ability to induce CD4 and CD8 regulatory T cells (Treg) (Gonzalez-Rey and Delgado, 2007b; Gonzalez-Rey et al., 2007a), and the generation of Treg cells has been found to play a major role in the beneficial effect of neuropeptides in several models of autoimmune diseases, *i.e.* experimental autoimmune encephalomyelitis (EAE), experimental colitis and models of rheumatoid arthritis (RA). Neuropeptides induce peripheral expansion of new antigen-specific CD4⁺CD25⁺ forkhead box P3 (FoxP3)⁺ Treg cells which suppress the activity of self-reactive T cells (Gonzalez-Rey and Delgado, 2007b; Gonzalez-Rey et al., 2007a; Nishida and Taylor, 1999). Most of the neuropeptides, with the exception of αMSH, generate CD4⁺CD25⁺ Treg cells from the CD4⁺CD25⁻ T-cell repertoire (Gonzalez-Rey and Delgado, 2007b; Gonzalez-Rey et al., 2007a). In contrast, αMSH seems to expand the natural Treg cells (Taylor and Namba, 2001).

The suppression of self-reactive effector T cells by neuropeptide-induced Treg involves direct cellular contacts dependent on CTLA-4 and/or the production of the immunosuppressive cytokines IL-10 and/or TGFβ. The involvement of Treg cells in the beneficial effect of neuropeptides in autoimmunity is supported by the fact that the *in vivo* blockade of the Treg cell mediators CTLA-4, IL-10 and TGFβ1 significantly reversed their therapeutic action. Based on the existing data, we propose that neuropeptides induce the generation of self-peptides-

specific Treg cells from otherwise conventional T cells, and that the newly recruited Treg prevent the progression of autoimmune diseases by suppressing systemic autoantigen-specific T and B cell responses as well as tissue-localized inflammatory responses.

6. Therapeutic action of neuropeptides in inflammatory disorders

The neuropeptide capacity to regulate a wide spectrum of inflammatory mediators, to switch the T_H1/T_H2 balance in favor of T_H2 immunity, and to contribute to the generation and/or activation of Treg makes them attractive therapeutic candidates for the treatment of inflammatory disorders and/or T_H1 -type autoimmune diseases. Treatment with VIP, α MSH, urocortin, adrenomedullin, cortistatin or ghrelin was shown to decrease the frequency, to delay the onset, and to reduce the severity of disease in experimental models of sepsis, collagen-induced arthritis, inflammatory bowel disease, type I diabetes mellitus, multiple sclerosis, Sjogren's syndrome, pancreatitis and uveoretinitis (reviewed in Gonzalez-Rey et al., 2007a; Taylor and Kitaichi, 2008). The therapeutic effects of these neuropeptides are associated with a reduction in the early phase of the disease associated with the initiation and establishment of autoimmunity to self-tissue components, as well as in the later disease phase associated with destructive inflammatory responses. The neuropeptides inhibit development of self-reactive T_H1 cells, their entry into target organs, the release of pro-inflammatory cytokines and chemokines, and the subsequent recruitment and activation of macrophages and neutrophils. This results in decreased production of destructive inflammatory mediators (cytokines, nitric oxide, free radicals and matrix metalloproteinases) by infiltrating and resident inflammatory cells. In addition, inhibition of the self-reactive T_H1 responses leads to decreased titers in IgG2a autoantibodies, an antibody subtype that activates complement and neutrophils and contributes to tissue destruction.

Two recent studies reported an additional and important mechanism in the action of some of these neuropeptides in inflammatory disorders. Using various models of endotoxic shock, sepsis and rheumatoid arthritis, we have found that the administration of urocortin, VIP or ghrelin decreased systemic levels of HMGB1 (Chorny and Delgado, 2008; Chorny et al., 2008), a DNA-binding factor that acts as a late inflammatory factor critical for the progression of sepsis and other inflammatory diseases (Lotze and Tracey, 2005). Macrophages are the major target with neuropeptides blocking HMGB1 cytoplasmic translocation and subsequent secretion (Chorny and Delgado, 2008; Chorny et al., 2008).

7. Neuropeptides as natural antimicrobial peptides: a new emerging field for ancient mediators

Based in the immunosuppressive activity of neuropeptides, the obvious question is whether they are affecting immunity against pathogens, with the expectation that the neuropeptide-treated subjects will be more susceptible to infectious diseases. Indeed, although arthritic mice treated with VIP were not significantly affected by a first infection with *Candida albicans*, they became more susceptible to reinfection, showing increased kidney colonization and suppressed anti-*Candida* IgG antibody production (Zafirova et al., 2004). However, interesting data arose from studies of experimental sepsis, a polymicrobial-induced systemic inflammatory model. VIP, urocortin and ghrelin protected against mortality in septic animals by downregulating early and late inflammatory mediators, and also reduced the *in vivo* peritoneal bacterial load (Chorny and Delgado, 2008). This unexpected finding could result from neuropeptide anti-bacterial activity. Recent studies showed indeed that α MSH, ghrelin, VIP and adrenomedullin act as potent antimicrobial peptides that directly kill various bacterial and yeast strains (Allaker et al., 2006; Brogden et al., 2005; Chorny and Delgado, 2008; Cutuli et al., 2000). The neuropeptides share some important characteristics with natural antimicrobial peptides, including small size (<10 kDa), high positive charge, and amphipathic α -helix structures

adopted upon interaction with membranes. Most data show that these neuropeptides could interact with the negatively charged outer leaflet of the plasma membranes of bacteria and insert into the cell membrane. This would lead to a rapid loss of the cell homeostasis and to pathogen death following membrane disruption (Allaker et al., 2006; Brogden et al., 2005; Chorny and Delgado, 2008). Therefore, as other antimicrobial peptides, neuropeptides could be considered integral components of the innate defense.

8. Are endogenous neuropeptides involved in immune tolerance in vivo?

The next few years will establish whether neuropeptides can be useful for clinical application in inflammatory disorders (see next section). However, a question that can be already answered is whether the endogenously produced neuropeptides are involved in immune homeostasis. Do neuropeptides act as natural tolerogenic factors? The fact that increased production of endogenous anti-inflammatory neuropeptides occurs in response to exacerbated inflammatory responses may be physiologically relevant (Gonzalez-Rey et al., 2007a; Varela et al. 2007). Noteworthy is also the presence of elevated amounts of these neuropeptides in barrier organs such as skin and mucosal barriers of the gastrointestinal, genital and respiratory tracts, suggesting that they may be key components of the innate immune system. This is also in agreement with their proposed antimicrobial activity. The relevance of neuropeptides as natural anti-inflammatory factors is supported by data obtained from several experimental inflammatory models performed in animals that are deficient in neuropeptides or their receptors. For example, mice that lack VIP show higher systemic inflammatory responses and are more susceptible to septic shock (Szema et al., 2006). Moreover, mice deficient or overexpressing VIP receptors show increased T_H1 - or $TH2$ -type responses, respectively (Goetzl et al., 2001; Voice et al., 2003). Regarding human conditions, an altered expression of VIP receptors in T cells has been related to aberrant T_H1 immunity in patients with multiple sclerosis and rheumatoid arthritis (Delgado et al. 2008c; Juarranz et al., 2008; Sun et al., 2006). Importantly, a genetic association between multiple-marker haplotypes of VIP receptor and susceptibility to suffer rheumatoid arthritis has been recently described (Delgado et al., 2008c). We reported that monocytes and synovial cells from arthritic patients bearing a certain VPAC1 polymorphism express less VIP receptors and respond less to VIP in comparison to cells from healthy subjects (Delgado et al., 2008c). Finally, there is correlation between reduced levels of VIP and high amounts of autoantibodies with VIPase activity in patients with lupus and autoimmune thyroiditis (Bangale et al., 2003). All these findings support the notion that reduced levels of neuropeptides and deficiencies and/or mutations in their receptors increase susceptibility to certain inflammatory and autoimmune diseases.

9. Are neuropeptides ready for the clinic?

The findings reviewed above indicate that these neuropeptides act in a pleiotropic and in many cases redundant manner to regulate the balance between pro-inflammatory and anti-inflammatory factors and between autoreactive T_H1 and Treg cells. Based on these characteristics, anti-inflammatory neuropeptides are prospective therapeutic agents for the treatment on autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis or Crohn's disease. Neuropeptides could serve as a platform to develop novel therapeutics that will block factors that initiate and drive inflammation and also restore immune tolerance.

One potential strategy is the direct administration of neuropeptides or neuropeptide analogs to patients. In addition to their wide spectrum of action, the molecular structure and size make neuropeptides attractive compounds. As small molecules, they possess excellent permeability properties that permit rapid access to the site of inflammation. This is critical for neuroinflammatory disorders, where the blood-brain-barrier is partially disturbed. The second

advantage owes to their high-affinity binding to specific receptors. Finally, the *in vitro* synthesis of neuropeptides is straightforward and permits easy modification if necessary.

Potential side effects arise from the effects of various neuropeptides on blood vessels, GI tract, reproductive organs, circadian rhythm, and CNS function. It is, however, important to note that some of these neuropeptides have been already tested in humans for the treatment of sepsis and other disorders (Varela et al., 2007), without such complications. This suggests that they might be well tolerated in doses similar to those that prevent inflammatory/autoimmune diseases in animals.

Despite the potential advantages of using neuropeptides in immune disorders, several obstacles stand between translating neuropeptide based-treatment into viable therapies in the clinic. Due to their natural structural conformation, neuropeptides are very unstable and extremely sensitive to tissue and plasma peptidases. Several strategies have been developed to increase their half-life, such as modifications or substitutions of certain amino acids or structure cycling to increase stability. Moreover, different strategies to improve neuropeptide delivery to target tissues and cells while protecting against degradation are now under investigation, including neuropeptide gene delivery or insertion into micelles or nanoparticles (Delgado et al., 2008b; Gonzalez-Rey and Delgado, 2007a). Other methods include combining neuropeptides with endopeptidase inhibitors to reduce the degradation of the peptide in circulation or administration of serum specific neuropeptide binding proteins (*i.e.* adrenomedullin binding protein-1) (Miksa et al., 2007). Other strategies, such as combining neuropeptides with phosphodiesterase inhibitors, aim to take advantage of the fact that most anti-inflammatory neuropeptides signal through the cAMP/PKA pathway (Gonzalez-Rey and Delgado, 2007a).

Development of metabolically stable analogs represents a prerequisite for translational applications. Understanding of the structure/function relationship of neuropeptides and their specific receptors, including receptor signalling, internalization and homo/heterodimerization, will facilitate the development of novel pharmacologic agents. An attractive strategy is the development of nonpeptide receptor agonists. However, in the case of the type 2 GPCRs, including VIP, urocortin, α MSH and adrenomedullin receptors, the pharmaceutical industry has so far failed to generate effective nonpeptide-specific agonists (Blakeney and Fairlie, 2005). Even where synthetic agonists were designed specifically for VIP receptors, they were less effective than the natural peptide in terms of anti-inflammatory activity (Delgado et al., 2004). Regarding type 1 GPCRs, the generation of several somatostatin agonists offered new therapeutic opportunities for the treatment of acromegaly and endocrine tumors (Weckbecker et al., 2003). However, the somatostatin receptor agonist octreotide was less effective than cortistatin in affecting inflammatory and autoimmune responses (Gonzalez-Rey and Delgado, 2007a; Weckbecker et al., 2003). Some recently developed nonpeptide ghrelin-receptor agonists remain to be tested.

A second potential translational strategy is the neuropeptide-mediated development of *in vivo* or *ex vivo* Treg. A considerable effort has been focused recently on the use of antigen-specific Treg cells generated *ex vivo* to treat autoimmune diseases, transplantation and asthmatic disorders (Bluestone, 2005). However, the ability to translate the use of Treg cells to the clinic has been limited by several problems, including the low frequency of these cells and their general immunosuppressive effects. A possible solution is the *in vitro* expansion of antigen-specific Treg using selected antigens. However, although Treg cells replicate relatively efficiently *in vivo*, they are anergic and refractory to stimulation *in vitro*. The capacity of VIP-induced tolerogenic DCs pulsed with self-antigens to induce antigen-specific Treg makes them attractive for the expansion/generation of antigen-specific Treg cells *ex vivo*, or alternatively, for their use as therapeutic cells that induce Treg *in vivo* (Gonzalez-Rey and Delgado, 2007b). One of the most important issues that need to be solved for this cell-based therapy is

to identify the antigen specificity in various autoimmune disorders. Whereas polyclonal Treg cells might function in allograft transplantation and in autoimmunity in lymphopenic (*i.e.* SLE) or inflammatory bowel disease, in other autoimmune disorders, antigen-specific Treg cells are most effective (Bluestone, 2005).

Regardless of the various technical and theoretical issues that still have to be solved for a successful transition to the clinical setting, the importance of neuropeptides in maintaining and reestablishing tolerance in animal models represents a significant development in autoimmunity research. The writer and activist Helen Keller said: "The highest result of education is tolerance". The recent findings elucidating the immunomodulatory actions of neuropeptides not only contribute to a better understanding of the dialogue between the immune and neuroendocrine system, but also permitted scientists to appreciate that neuropeptides play a critical role in educating the immune system to remain tolerant.

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Table 1

Anti-inflammatory neuropeptides: immune sources, receptors and effects

Neuropeptide	Peptide Family ^a	Immune source ^b	Receptor ^c		Immune Function ^d
			Type	Expression in immune system	
VIP	PACAP secretin glucagon GHRH	CD4 Th2, CD8, PMN, VPAC1 Mast cells	VPAC2 PAC1	T, Mφ, Mo, DC, PMN T, Mφ (after activation) Mφ, Mo	↓ inflammatory cytokines: TNFα, IL-12, IL-6, IL-18, MIF, IL-1β ↓ iNOS and COX2 expression ↓ chemokine production: IL-8, Rantes, IP-10, MIP-1α, MIP-2, MCP-1 ↓ costimulation Mφ and DCs ↓ HMGB1 secretion ↑ IL-10, IL-1Ra production ↑ T cell proliferation ↓ TLR expression ↑ T _H 1 response: IL-2 and IFNγ production ↑ T _H 2 response: IL-4 and IL-5 ↑ regulatory T cells ↑ generation tolerogenic DCs Antimicrobial activity
αMSH	POMC ACTH	T, Mo, DC	MC1R MC3R MC4R MC5R	T, Mφ, Mo, DC, PMN, NK, B Mφ, Mo - T, B	↓ inhibits TNFα, IL-6, IL-1β, iNOS expression ↓ antigen presentation and costimulation by DCs ↓ TLR expression ↑ IL-10 production ↑ T cell proliferation Induces regulatory T cells ↑ generation tolerogenic DCs Antimicrobial activity
Urocortin	CRH urotensin sauvagine	T, B, Mφ, Mo, Mast cells	CRHR1 CRHR2	- T, Mφ, Mo, DC, PMN	↓ inflammatory factors: TNFα, IL-12, IL-18, MIF, NO, IL-6, IL-1β ↓ chemokines: IP-10, Rantes, MIP-1α, MIP-2, MCP-1, eotaxin ↑ IL-10 production ↑ HMGB1 secretion ↑ macrophage apoptosis ↑ T cell proliferation ↓ T _H 1 response: IL-2 and IFNγ production Induces regulatory T cells
AM	calcitonin CGRP amylin	Mφ, Mo	CRLR-RAMP1 CRLR-RAMP2 CRLR- RAMP3	- T, Mφ, Mo, DC T, Mφ, Mo, DC	↓ inflammatory factors: TNFα, IL-12, IL-6, IL-18, MIF, NO, IL-1β ↓ chemokines: Rantes, IP-10, MIP-1α, MIP-2, MCP-1, eotaxin ↑ IL-10 production ↑ T cell proliferation ↑ PPAR expression in Mφ ↓ T _H 1 response: IL-2 and IFNγ production Induces regulatory T cells Antimicrobial activity
Cortistatin	SOM	T, Mo, Mφ	sst1-5 GHSR	T, Mφ, Mo, DC T, Mφ, Mo, DC	↓ inflammatory factors: NO, TNFα, IL-12, IL-6, IL-18, MIF, IL-1β ↓ chemokines: Rantes, IP-10, MIP-1α, MIP-2, MCP-1, eotaxin ↑ IL-10 production ↑ T cell proliferation ↓ T _H 1 response: IL-2 and IFNγ production Induces regulatory T cells
Ghrelin	motilin	Mo, Mφ	GHSR	T, Mφ, Mo, DC	↓ inflammatory factors: NO, TNFα, IL-12, IL-6, IL-18, MIF, IL-1β

Neuropeptide	Peptide Family ^a	Immune source ^b	Receptor ^c		Immune Function ^d
			Type	Expression in immune system	
					↓ chemokines; Rantes, IP-10, MIP-1α, MIP-2, MCP-1, eotaxin ↑ IL-10 production ↓ HMGB1 secretion ↓ T cell proliferation ↓ T _H 1 response; IL-2 and IFN γ production Induces regulatory T cells Antimicrobial activity

^aFamily of peptides showing some homology in sequence/structure with the referenced neuropeptides.

^bImmune cells that produce anti-inflammatory neuropeptides.

^cExpression of different receptors. VPAC1, VPAC2, PAC1, CRLR and CRFR belong to type 2 of G-protein-coupled receptors (GPCR). GHSR, MC1-5R, and sst1-5 belong to type 1 GPCR family.

^dMajor roles of the neuropeptides related to the inflammatory and autoimmune response. ↑ stimulation; ↓ inhibition.

Abbreviations: AM, adrenomedullin; SOM, somatostatin; MC1-5R, melanocortin receptors 1-5; GHSR, ghrelin receptor; CRLR, calcitonin-related ligand receptor; CRHR, CRH receptor; VPAC, VIP/PACAP receptor; RAMP, receptor-activity-modifying proteins; GHSR, growth hormone-secretagogue receptor; T, T cells; Mφ, macrophage; Mo, Monocyte; DC, dendritic cell; PMN, polymorphonuclear cell; B, B cells; NK, natural killer cells; TNF-α, tumour necrosis factor-α; IP-10, interferon-γ inducible protein-10; IFN, interferon; MIF, macrophage migration inhibitory factor; IL, interleukin; MIP, macrophage inhibitory protein; MCP, macrophage chemoattractant protein; NO, nitric oxide; iNOS, inducible nitric oxide synthase; COX2 cyclooxygenase-2; IL-1Ra, IL-1 receptor antagonist; T_H, T helper cell; HGMB1, high mobility group box 1; PPARγ, peroxisome proliferator-activated receptor-γ; TLR, Toll-like receptor.

(Adapted from Varela et al., 2007; Gonzalez and Delgado, 2007a)