Inhibitory neuropeptide receptors on macrophages

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ABSTRACT — The immune response, both in innate and adaptive immunity, is controlled at several levels, including signaling from the central nervous system. Neuropeptides released within the lymphoid organs modulate the immune response, either as stimulators or inhibitors. The subject of this review is the description of macrophage-expressed receptors of inhibitory neuropeptides. We describe the inhibitory effects on macrophage function for several neuropeptides, the receptors that mediate those activities, and the molecular mechanisms initiated by some of these receptors in terms of transduction pathways and transcriptional factors. © 2001 Éditions scientifiques et médicales Elsevier SAS

1. Neuropeptide sources in the lymphoid organs

Two different neuropeptide sources exist in the lymphoid organs, i.e. the peptidergic innervation and the immune cells themselves. In addition, some of the neuropeptides can be released from the hypothalamic-pituitary axis as hormones or prohormones and arrive in the lymphoid organs via the circulation. A number of studies established and characterized the peptidergic innervation in the lymphoid organs, with neuropeptide Y (NPY) colocalized with norepinephrine (NE) in the sympathetic fibers, substance P (SP) and calcitonin gene-related peptide (CGRP) closely overlapping anatomically, but not necessarily colocalized in the sensory innervation, and vasoactive intestinal peptide (VIP) present in the cholinergic innervation [1–3]. In addition, a less abundant galanin (Gal), somatostatin (Som), and opioid innervation was also reported [1–4]. The peptidergic terminals were shown to be in anatomical apposition to the immune cells, particularly to macrophages.

Various immune cells also express and secrete neuropeptides. Here we will review only the macrophage/monocyte neuropeptide production. Macrophages from various organs and tissues were shown to express mRNA and secrete SP, corticotropin-releasing factor (CRF), proopiomelanocortin (POMC)-derived peptides such as alphamelanocyte stimulating factor (α-MSH), ACTH, and β-endorphins (End), Som, CGRP, NPY, and atrial natriuretic peptide (ANP) [5–14]. Stimulation with LPS, cytokines, or in the case of thymic macrophages with dexamethasone, leads to increased neuropeptide production [6, 8, 10–14].

2. General effects of neuropeptides on macrophage activation and functions

Numerous studies reported on the effects of neuropeptides on macrophage function in vivo and in vitro. Table I summarizes the neuropeptide effects on macrophage phagocytosis, adherence/chemotaxis, cytokine production, production of nitric oxide and oxygen radicals and antigen presentation. Whereas some neuropeptides such as SP, PRL, NT and NPY stimulate most macrophage functions, others act as inhibitors especially for the production of proinflammatory cytokines and nitric oxide/oxygen radicals. Since the focus of this review is on inhibitory neuropeptide receptors, we will discuss only the neuropeptides with well established inhibitory effects on macrophage function in either innate or adaptive immunity.

3. Inhibitory neuropeptide receptors on macrophages

3.1. Melanocortin receptors (MCRs)

The POMC gene product is processed in a tissue-specific manner giving rise to a variety of peptides, including ACTH1-39, α-MSH and β-endorphins. As mentioned
that mediates the inhibitory effect of macrophage cell line Raw 264.7 also expresses MC1-R with MC3-R and MC5-R, with MC1-R mediating the inhibition of chemotaxis by stimulated neutrophils express MC1-R that mediates the chemokine release by ACTH-related peptides MC3-R that mediates the inhibition of phagocytosis and inhibition of chemotaxis Cytokines and/or oxygen radicals Antigen presentation References

<table>
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<tr>
<th>Neuropeptide</th>
<th>Macrophage treatment</th>
<th>Phagocytosis</th>
<th>Adherence/chemotaxis</th>
<th>Cytokines</th>
<th>Nitric oxide and/or oxygen radicals</th>
<th>Antigen presentation</th>
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<tbody>
<tr>
<td>NT PRL SP</td>
<td>resting LPS or resting</td>
<td>increase</td>
<td>increase</td>
<td>↑IL-1, IL-6 ↑IFN-γ, IL-10 ↑TNF-α, IL-12 ↓TGF-β1</td>
<td>increase increase</td>
<td>↑MHCII</td>
<td>[15] [16] [17–19]</td>
</tr>
<tr>
<td>NPY Opioids</td>
<td>BetaEND LPS, GM-CSF stimulation</td>
<td>increase</td>
<td>increase</td>
<td>↑IL-1, IL-10 ↑IL-6, TNF-α ↑TNF-α ↑IL-12p35</td>
<td>inhibition inhibition</td>
<td>↓B7.2 ↓MHCII ↓stimulation of T cells</td>
<td>[20] [21, 22]</td>
</tr>
<tr>
<td>ACTH ANF CGRP</td>
<td>LPS LPS, IFN-γ, GM-CSF inhibition</td>
<td>↓TNF-α, IL-1β ↓IL-1, TNF-α ↓IL-12 p40 ↓IL-6, IL-10</td>
<td>↓TNF-α, IL-6, IL-10</td>
<td>↓IFN-γ</td>
<td>↓IL-6, TNF-α</td>
<td>↓IFN-γ, TGFβ ↑IL-10</td>
<td>[17, 24, 25] [26–28] [29, 30]</td>
</tr>
<tr>
<td>SOM SOM CRF αMSH VIP/PACAP</td>
<td>LPS ± IFN-γ inhibition</td>
<td>↓TNF-α, IL-1 ↓IL-10</td>
<td>↓TNF-α, IL-6 ↓IL-12p40 ↓IFN-γ, TGFβ ↑IL-10</td>
<td>↓IL-6, TNF-α</td>
<td>↓IFN-γ</td>
<td>↓B7.2/B7.2</td>
<td>[33] [34, 35] [36–41]</td>
</tr>
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above, macrophages are capable to express the POMC gene, and to process the translated product leading to the secretion of α-MSH and β-endorphins [6, 9]. ACTH and especially α-MSH have potent direct anti-inflammatory activities, in addition to the induction of glucocorticoids by ACTH via the hypothalamic-pituitary-adrenal axis. Both ACTH and α-MSH bind to a class of closely related, cAMP-inducing, G-protein-coupled receptors, called the MCRs. In contrast to MC1-R and MC2-R that are expressed primarily in melanocytes and adrenal cortex, respectively, the other three receptors are widely distributed both in brain and the periphery. The expression of MCRs has been demonstrated in both macrophages and neutrophils, and in macrophage cell lines. Peritoneal macrophages express MC3-R that mediates the inhibition of phagocytosis and chemokine release by ACTH-related peptides [24], and stimulated neutrophils express MC1-R that mediates the inhibition of chemokine release by α-MSH [42]. The murine macrophage cell line Raw 264.7 also expresses MC1-R that mediates the inhibitory effect of α-MSH on iNOS expression and subsequent nitric oxide production [34], and the human monocytic THP-1 cells express MC1-R, MC3-R and MC5-R, with MC1-R mediating the inhibition of TNF-α by α-MSH [43].

3.2. Atrial natriuretic peptide (ANP) receptors

The natriuretic peptide family consists of three members, ANP and BNP (brain natriuretic peptide), both of cardiac origin, with endocrine functions, and CNP (C-type natriuretic peptide), produced mostly in brain where it plays a paracrine role. Peritoneal, bone marrow-derived, and thymic macrophages produce all three natriuretic peptides, and macrophage stimulation has a regulatory effect, increasing ANP and particularly CNP production, and decreasing BNP expression [14]. Three receptors have been identified for the natriuretic peptide family. NPR-A and NPR-B (which favor ANP and CNP binding, respectively) are coupled to guanylate cyclase and lead to intracellular cGMP accumulation. In contrast, NPR-C serves as a clearance receptor which binds and internalizes natriuretic peptides from blood but does not stimulate the guanylate cyclase. Macrophages (peritoneal, bone marrow-derived, cell lines such as Raw 264.7 and J774) express all three receptors, but only ANP inhibits TNF-α, IL-1β and nitric oxide production through the induction of cGMP [28]. Nitric oxide production is affected through a reduction in NF-κB binding to its transactivating site in the iNOS promoter [26], and the inhibition of TNF-α and IL-1β by ANP is mediated by NPR-A and reduction in both NF-κB and AP-1 binding [28].

3.3. CGRP receptors

The primary transcript for calcitonin is processed to calcitonin mRNA in thyroid C cells, and to CGRP mRNA in brain and the peripheral nervous system. Macrophages, specifically Langerhans cells, also express CGRP [12]. Based on recognition of different antagonists, two types of CGRP receptors have been identified, CGRP1 and CGRP2.
receptors. The transduction pathways used by the CGRP receptors depend on their localization, with most receptors in the periphery using the adenylate cyclase pathway to activate cAMP. Macrophages express CGRP receptors, and in most cases the effects of CGRPs are mediated through the CGRP1 receptor. For example, the potentiation of IL-6 production, and the inhibition of TNF-α production and of the antigen-presenting function, are mediated through the CGRP1 receptors and the cAMP/PKA pathway [44]. Dendritic cells, both immature and mature, also express mRNA for CGRP1 receptor that mediates the reduction in B7.2, MHC class II, and in the stimulation of allogeneic T cells. These effects, however, appear to be dependent on Ca2+ mobilization rather than cAMP induction [45]. Actually, Parameswaran et al. [46] reported recently that cells transfected with the CGRP1 receptor respond to CGRPs by increases in cAMP, ERK and p38 MAPK activity. Activation of ERK is induced by either cAMP or PI3-kinase, whereas activation of p38 is entirely cAMP-dependent. Therefore, both cAMP and Ca2+ mobilization might be required for an optimal response to CGRPs through the CGRP1 receptor.

3.4. Somatostatin receptors

Somatostatins were isolated from hypothalamus, as well as from pancreatic and gastrointestinal tissues. In addition, activated macrophages and macrophage cell lines express pre-pro-SOM mRNA and synthesize the mature peptide [11, 47]. In vivo, SOM was induced in macrophages from popliteal lymph nodes following LPS, IFN-γ, or LPS plus IFN-γ injections [10]. Five different types of SOM receptors have been identified to date. All are coupled to G proteins and inhibit adenylate cyclase and Ca2+ fluxes. In peritoneal macrophages, functionally active concentrations of SOM progressively increase cGMP levels [48]. Although mRNA for all SOM receptors was identified in various lymphoid tissues, Sst2A appears to be the prevalent receptor in thymic macrophages, granuloma macrophages from schistosome-infected mice and from patients with sarcoidosis, and in synovial macrophages from patients with rheumatoid arthritis. SOM inhibits the production of a number of pro-inflammatory agents, such as TNF-α, IL-6, IFN-γ and nitric oxide [10, 31, 32]. Although the mechanisms by which SOM acts as an anti-inflammatory agent are poorly understood, SOM exerts both a direct effect on the immune cells and an indirect one through glucocorticoids. A recent report [49] demonstrates that SOM does not upregulate the glucocorticoid receptors (GRs) on macrophages, but increases glucocorticoid binding and signaling by stabilizing the GR-associated induced heat shock protein Hsp90. This stabilization is mediated through a reduction in calpain activity.

3.5. CRF receptors

CRF is widely distributed in the CNS, particularly in the hypothalamic nuclei, as well as in various organs in the periphery, including thymus and spleen. Macrophages represent the major CRF-positive cells in the immune organs [7]. CRF inhibits both T-cell proliferation and the secretion of TNF-α and IL-1β by activated macrophages [33]. Three receptor subtypes, CRF1, CRF2a and CRF2b, have been identified in brain, pituitary and spleen, where they are restricted to macrophage-rich regions. The CRF receptors are G-protein-coupled, and induce intracellular cAMP. In Kupffer cells, urocortin, a peptide homologous to CRF, was shown to inhibit TNF-α production directly, in a corticosteroid-independent manner, through cAMP increases [33].

3.6. VIP (vasoactive intestinal peptide) and PACAP (pituitary adenylate cyclase-activating polypeptide) receptors

The effects of VIP and PACAP on immune cells, and specifically on macrophages, are well documented. In addition to the VIP-ergic innervation of the lymphoid organs, activated T cells, and particularly Th2 effectors are the major VIP sources in the immune system ([50]; see notes added in proof (1)). VIP and PACAP affect many levels of the immune response in vivo and in vitro, from macrophage-mediated innate immunity, to antigen presentation, proliferation and differentiation of T cells, activation-induced T-cell apoptosis, and CD8+ T-cell cytotoxicity [36, 51–54]. Here we will review only the effects on activated macrophages. VIP and PACAP act as general anti-inflammatory agents, inhibiting the production of proinflammatory cytokines such as TNF-α, IL-12, IL-6 and the induction of iNOS, and stimulating the production of the anti-inflammatory cytokine IL-10 [37–41]. In vivo, VIP and PACAP exert similar effects, and protect mice in a high endotoxemic model of septic shock (reviewed in [51]). In addition to the effects on cytokine and nitric oxide production, VIP and PACAP also reduce the expression of the costimulatory molecules B7.1 and B7.2 in activated macrophages, and inhibit their costimulatory activity for naïve T cells [36]. The effects of VIP and PACAP are mediated through specific receptors. Three receptors, VPAC1, VPAC2 and PAC1 have been cloned and characterized [55]. VPAC1 and 2 bind both VIP and PACAP with similar affinity, and are coupled primarily to the cAMP/PKA pathway. PAC1 binds PACAP with an affinity 300–1 000-fold higher than VIP, and is coupled to both adenylate cyclase and phospholipase C. Studies with murine peritoneal macrophages and macrophage cell lines and with the human monocytic cell line THP-1 indicate that VPAC1 and PAC1 are constitutively expressed, whereas VPAC2 is induced upon activation ([39, 56] and unpublished results). In both peritoneal macrophages and the Raw 264.7 cells, VIP and PACAP affect TNF-α, IL-10, NO, IL-12p40 and B7 expression primarily through VPAC1. In contrast, the inhibition of IL-6 is mediated through PAC1 (reviewed in [51]). Extensive studies have been done on the molecular mechanisms involved in the inhibition of TNF-α, IL-12p40, IL-10 and iNOS expression by VIP/PACAP. The stimulatory effect on IL-10 transcription is cAMP-dependent, and mediated through an increase in CREB (cAMP-regulatory element binding protein) [41]. In contrast, the VIP/PACAP inhibition of TNF-α, iNOS and IL-12p40 expression occurs through two transduction pathways, a cAMP-dependent and a cAMP-independent pathway. For TNF-α, the cAMP independent pathway leads to a reduction in NF-κB binding and the cAMP-dependent pathway results in a change
in the composition of the CRE-binding complex from high-c-Jun/low-CREB to high-CREB/low-c-Jun [57]. Optimal activation of iNOS and IL-12p40 requires both LPS and IFN-γ. For these two genes, VIP and PACAP inhibit IRF-1 binding in a cAMP-dependent manner and NF-κB binding in a cAMP-independent manner [40, 56]. Recently, we showed that the inhibition of IRF-1 binding is due to a reduction in IRF-1 transcription mediated through the inhibition of Jak1/2-STAT1 phosphorylation/activation [58] (figure 1). Also, we demonstrated that the VIP/PACAP promotes JunB and inhibits c-Jun phosphorylation through effects on the MEKK1/MEK4/JNK pathway [59] (figure 2). Replacement of c-Jun with JunB in transcriptional complexes results in the transcriptional inactivation of various cytokine promoters. Finally, VIP/PACAP affects sNF-κB at various levels (figure 3). A cAMP-independent pathway is responsible for the stabilization of I-κB, the cytoplasmic inhibitor complexed to NF-κB. The stabilization of I-kB is due to the inhibition of I-kB phosphorylation by IKKe, and results in the cytoplasmic sequestration of p65, a major component of the NF-κB complex [56]; see notes added in proof (2)). In addition, VIP/PACAP promotes CREB phosphorylation through the cAMP/PKA pathway, and phosphorylated CREB translocates to the nucleus where it competes for the coactivator CBP (CREB-binding protein). CBP, found in limiting amounts in the nucleus, is necessary for the stabilization of a transcriptionally active complex through interactions with several DNA bound transcriptional factors (figure 3). Removal of CBP by CREB prevents the transcriptional complex from acquiring the ‘transcriptionally active’ conformation. Finally, VIP/PACAP also affect the phosphorylation of TBP (the TATA-box binding protein) through the inhibition of the MEKK1/MEK3/6/p38 MAPK pathway (see notes added in proof (2)). In the absence of phosphorylated TBP there is an inefficient recruitment of the RNA polymerase II, which further weakens transcription.

Although cAMP-inducing agents mimic several of the VIP/PACAP effects, the fact that these two neuropeptides act through both cAMP-dependent and cAMP-independent pathways allows them to function as extremely efficient immunomodulators.

4. Conclusions

Through their participation in both innate and adaptive immunity, macrophages play a crucial role in the defense against pathogens. Macrophage development and activa-
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Figure 3. VIP and PACAP affect NF-κB transcriptional activity at multiple levels. VIP and PACAP bind to the VPAC1 receptor on macrophages and activate both the cAMP/PKA pathway, and a cAMP-independent pathway. The cAMP-dependent pathway stabilizes IκB by inhibiting the kinase activity of IKKα. The stabilized IκB sequesters the p65/p50 complexes in the cytoplasm. This results in a decreased NF-κB binding to promoters. The cAMP-dependent pathway phosphorylates CREB, leading to its nuclear translocation and subsequent binding to CBP. In the absence of the coactivator CBP, the transcriptional complexes are not fully active. In addition, by inhibiting the MEKK1/MEK3/6/38 pathway, VIP and PACAP reduce the phosphorylation of TBP (the TATA-box binding protein) resulting in a reduced recruitment of RNA polymerase II.

Notes added in proof


References


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