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MODULATION OF IMMUNE FUNCTION BY INTESTINAL NEUROPEPTIDES

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Abstract

Direct regulatory control of the immune system by the central nervous system has been postulated. In support of this view is a large body of literature describing immunoregulatory activities of neuropeptides isolated from the gastrointestinal tract. In this review we examine the evidence for expression of specific receptors for gut peptides on immune effector cells and further explore the regulatory effects of these peptides on immune function. Peptides to be discussed include substance P, somatostatin, vasoactive intestinal peptide (VIP), the opioid peptides leu and met enkephalin, calcitonin gene related peptide (CGRP), neuropeptide Y, and cholecystokinin (CCK).

Key words: Neuropeptides, immunomodulators, neuroimmune axis.

Modulation of immune function by the central nervous system (CNS) was first suggested by early empirical descriptions of increased susceptibility to infectious diseases following emotional or physical stress. Later, more systematic investigations describing stress-induced alterations of immune function, as well as the immunoinhibitory effects of glucocorticoids provided a reasonable physiological basis for the earlier observations. Thus increased susceptibility to disease following stress could be explained solely (and somewhat naïvely) on the basis of neuroendocrine interactions resulting in elevated glucocorticoid levels and subsequent immunosuppression.

Although interaction of the CNS with the immune system can occur via several endocrine pathways, the presence of various neuropeptide receptors on cells of the immune system suggests more direct communication pathways. Moreover, immune cells release factors which can influence neuronal activity. Thus direct communication between the CNS and the immune system may well be bidirectional.

Due to its anatomical organization with abundant autonomic innervation, as well as its extensive lymphoid compartment, the gastrointestinal tract represents one potential site in which to study interactions between these two systems. Moreover, a number of neuropeptides with observed or potential immunomodulatory effects have been isolated from gut tissues. Those peptides most widely studied include substance P, somatostatin, vasoactive intestinal peptide and the opioid peptides, leu- and metenkephalins. In addition, more recent studies indicate that the peptides calcitonin gene-related peptide, neuropeptide Y, and cholecystokinin may also regulate the immune response. Distribution of these peptides within lymphoid tissues is summarized in the Table. The purpose of this review is to provide a brief survey of the current literature surrounding these peptides in the context of immune regulation.

Substance P

Substance P (SP) is an undecapeptide belonging to the tachykinin family of peptides. Tachykinins are characterized by their potent vasodilatory effects which result in marked hypotension and subsequent tachycardia. As a group the tachykinins share a common C terminal sequence phe-X-gly-leu-met-NH_2, where X represents a site of aromatic or aliphatic amino acid substitution (1).

Within the central nervous system, SP functions as a neurotransmitter, with discrete localization in several areas of the brain and spinal cord (2). In the gastrointestinal tract, nerve fibers containing SP are found within the perivascular nerve plexuses, particularly in association with small arterioles (3), suggesting a role in regulating

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Table

Location of gut neuropeptides (5, 63)

Peptide	Lamina propria	Peyer's patches	Lymph node	Spleen
Substance P	+	+	+	+
Somatostatin	+	+		
Vasoactive intestinal peptide	+	+	+	
Enkephalins				+
Calcitonin gene-related peptide	+	+	+	+
Neuropeptide Y			+	+
Cholecystokinin	+			+

intestinal blood flow. In addition, SP immunoreactivity has been detected in the myenteric plexuses of the gut wall, as well as in endocrine cells present in the mucosa (2). Aside from peptide distribution within the gastrointestinal tract, the widespread distribution of SP receptors within the gut, including vasculature, smooth muscle, epithelium, and lymphoid nodules (4) suggests the importance of this peptide in normal gut physiology. In the spleen, SP-containing neurons are abundantly located in both the red and white pulp, but only sparsely located in the periarteriolar lymphatic sheath, where T lymphoyctes are concentrated (5).

With regard to its role in modulating the immune response, the interactions of SP with a number of lymphocyte and accessory cell populations have been widely studied. Both T (6, 7) and B (6, 8, 9) lymphocytes express membrane receptors for SP. In the IM-9 human B lymphoblast cell line, the SP receptor has been characterized as a 38 kD glycoprotein, derived from 36 and 33 kD precursors following posttranslational glycosylation (10). Glycosylation is not, however, apparently required for peptide binding, since pretreatment of cells with tunicamyin fails to inhibit SP binding (10). Functional activity in these cells following SP binding, however, was not assessed. The SP receptor has recently been cloned from rat brain (11) and submandibular gland (12) and demonstrated to be a 46 kD protein belonging to the G-protein family of receptors.

The affinity with which the lymphocyte SP receptor binds its ligand, as assessed by Scatchard analysis of the IM-9 receptor, is high ($k_D = 0.3–0.87$ nmol/l) (7). Similar results have been reported for T cells ($k_D = 1.85$ nmol/l) (7). Based upon competitive inhibition studies utilizing related tachykinins (substance K and eledoisin), binding of SP to the lymphocyte membrane receptor occurs via the carboxy terminus (6, 9). Interestingly, SP binding to the IM-9 SP receptor downregulates receptor expression (6).

Functional studies provide additional support for the concept of an immunomodulatory function of SP. SP stimulates proliferation (13, 14) and acts as a comitogen in phytohemagglutinin (PHA) and conconavalin A (ConA) induced proliferation (14, 15), as assessed by ^{3}H-thymidine incorporation. In addition, SP augments pokeweed mitogen (PWM) induced interferon-γ production by human peripheral blood mononuclear cells (16).

With regard to B cell function, Stanisz et al. (15) demonstrated increased immunoglobulin synthesis in mitogen-pulsed lymphocyte populations treated with SP in vitro. This work is of particular interest from several perspectives. First, IgA production was much more enhanced than IgM or IgG. Secondly, cells derived from Peyer's patches were more sensitive to the effects of SP than cells derived from the spleen or mesenteric lymph nodes. Subsequent studies utilizing cells from mice implanted with osmotic pumps demonstrated SP-mediated enhancement of IgA synthesis in vivo (17).

In addition to lymphocytes, other cell populations related to immune function are also responsive to SP treatment. Early studies by Hartung & Toyka (18) demonstrated that treatment of guinea pig peritoneal macrophages with SP in vitro resulted in the production of peroxide, super oxide, and thromboxane B2. In later studies using standard radioligand binding techniques, this same group demonstrated the presence of SP receptors on guinea pig macrophages (19). Receptor affinity was somewhat lower ($k_D = 19$ nM) than that reported for lymphocytes, and both carboxy and amino terminals appeared to be relevant for ligand binding. In the case of human monocytes, SP treatment can increase cytotoxicity against tumor targets (20), as well as stimulate production of interleukin 1, interleukin 6, and tumor necrosis factor-α (21, 22). In addition, SP appears to be chemotactic for human monocytes (23).

Neutrophils are also responsive to treatment with SP. Increased metabolic activity (24–26), degranulation (24), and production of inflammatory mediators (24), have all been observed in populations of neutrophils following treatment with SP. In addition, SP-mediated stimulation of histamine release from mast cells and basophils has been reported in various species (27), although human mast cells and basophils fail to respond (27, 28).

Neuropeptides can potentially interact with phagocytic cells via a high affinity receptor specific for that peptide or via membrane structures important in phagocytosis (29) or chemotaxis (23). The carboxy terminal end of the peptide is required for effects of SP, which are mediated via the high affinity SP receptor (e.g. cytotoxicity and release of cytokines), whereas stimulation of the phagocytic or chemotactic mechanisms appears to involve the amino terminal tetrapeptide (29).

Somatostatin

Somatostatin (SS), in its most common form, exists as a 14 amino acid structure with disulfide linkage between 2

cysteine residues resulting in a cyclic molecule. It is widely distributed throughout the central nervous system (2, 30), particularly in the hypothalamus, from which it was first isolated. Its name is based upon its action on the anterior pituitary, where it acts to inhibit the release of growth hormone. Within the brain, SS is found in highest concentrations within the median eminence and arcuate nucleus, although it is also found in the cortex, particularly in the preoccipital region (30). In the peripheral nervous system, SS is associated with sensory fibers, often associated with catecholamine-containing fibers. In the gastrointestinal tract, SS is associated with small fibers in the mucosa, as well as neurons of the submucosal and myenteric plexuses (2, 3, 30).

Production of SS occurs via synthesis of a large precursor molecule, preprosomatostatin. Post translational cleavage results in the mature form, most commonly as the 14 amino acid form, but often as a 28 amino acid chain (30).

Like SP, the action of SS on various lymphocyte populations has been widely studied. Utilizing fluorescent labelled SS, Scicchitano et al. (31) demonstrated specific SS binding on murine Peyer's patch and splenic T and B lymphocytes. Their data indicated significant differences among the various populations tested, with T and B cells derived from Peyer's patches showing greater SS binding frequencies than those derived from the spleen. Moreover, in assessing B-cell binding with regard to surface immunoglobulin isotype, these authors reported that binding was greatest among Peyer's patch cells expressing either IgA or IgM. Results among splenic B cells were slightly different with a much higher proportion of IgA^+ cells binding SS than either IgM^+ or IgG^+.

Human lymphocyte expression of SS receptors has also been reported. In an early study, Bhathena et al. (32) reported binding of SS to enriched populations of peripheral blood lymphocytes. Human SS lymphocyte receptors were further characterized in a recent report by Sreedharan et al. (33) utilizing the Jurkat T cell tumor line and the U266 IgE-secreting myeloma. Data from this study indicated the presence of two populations of SS receptors on both cell lines. High affinity receptors ($k_D = 3-5$ pmol/l) were present in relative low density on both the T and B cell line (144 and 1 295 sites/cell respectively). Lower affinity receptors ($k_D = 66-100$ nmol/l) were expressed at a much higher density on both cell populations.

Although there are numerous reports regarding the alteration of lymphocyte function following treatment with SS, the effects reported are somewhat contradictory. Using a murine system, Stanisz et al. (15) and Scicchitano et al. (31) demonstrated inhibition of ConA induced lymphocyte proliferation and immunoglobulin secretion in splenic, mesenteric lymph node, and Peyer's patch lymphocytes following in vitro treatment with SS (10^{-8} mol/l). In contrast, Krco et al. (34) reported that murine lymph node cells stimulated with mitogens (PHA, ConA, and LPS) or alloantigen in vitro were not affected by similar concentrations of SS. Still other reports indicate that untreated (35, 36) or mitogen-stimulated (37) lymphocyte proliferation may be enhanced by in vitro SS treatment at comparable doses ($10^{-6}-10^{-18}$ mol/l).

In the case of human lymphocytes, the observed effects of SS on lymphocyte function appear to be more consistent. Using MOLT-4 lymphoblasts and PHA-stimulated human T cells, Payan et al. (38) demonstrated suppression of cell proliferation following SS ($10^{-13}-10^{-9}$ mol/l) treatment. Similarly, suppression of mitogen-induced proliferation has also been observed in cell populations derived from volunteers receiving intravenous infusion of SS (250 μg/h) (39). Additional in vivo suppressive effects of SS are suggested by a case report by Vehmeyer et al. (40) in which they describe the failure of PHA to promote colony growth of T cells isolated from a somatostatinoma patient.

In addition to its effects on lymphocytes, SS binds to human monocyte populations (32), and can alter functional activity of various monocyte/macrophage populations. Rat peritoneal macrophages exhibited enhanced antibody-dependent cell-mediated cytotoxicity against sheep erythrocytes following treatment with SS (41), which appeared to be dependent upon both increased Fc receptor expression and production of active oxygen species. Moreover, in vivo administration of SS enhances colloidal carbon clearance in rats, presumably through the action of activated hepatic Kupffer cells (42). In the case of human peripheral blood monocytes Peck (20) has reported partial inhibition of interferon-mediated cytolytic activity in populations of human monocytes. This effect could be abrogated by the addition of SP, suggesting both negative and positive regulation of this function by neuropeptides.

Finally, SS may also be involved in secretory activity of basophils. Goetzl et al. (43, 44) have demonstrated that histamine and leukotriene D4 release from human basophils sensitized with anti-human IgE can be inhibited by SS treatment ($3 \times 10^{-9}-10^{-13}$ mol/l). Moreover, inhibition in this context appears to involve only immune mediated release, since SS was ineffective in inhibiting ionophore mediated release. The significance of these findings relative to the potential role of SS in neurogenic regulation of basophil function, must be viewed with some caution, in that basophils, themselves, can produce an SS-like substance (45). Thus basophil degranulation may, in fact, be controlled in vivo by an autocrine or paracrine mechanism rather than through direct neurogenic control.

Vasoactive intestinal peptide

Vasoactive intestinal peptide (VIP), first isolated from porcine gastrointestinal tract (2), is a 28 amino acid peptide structurally related to a group of gastrointestinal regulatory compounds included in the secretin family.

Other members include PHI (polypeptide with amino-terminal histidine and carboxy terminal isoleucine), growth hormone releasing factor (GHRF), helodermin, secretin, glucagon, and GIP (gastric inhibitory peptide). Named on the basis of its marked vasodilatory effect, VIP is a strong smooth muscle relaxant. Thus it appears to regulate intestinal motility. In addition, water and ion secretion in the gut are also enhanced by VIP (2).

Although first isolated in the gut, VIP has been localized within the CNS in the cortex, hypothalamus, amygdaloid nucleus, and striatum (2). In the PNS, nerve fibers containing VIP are present in the superior and inferior mesenteric ganglia, as well as within the submucosal and myenteric plexuses in the gut. In addition, immunoperoxidase staining for VIP has also localized VIPergic fibers associated with the vasculature in the areas of Peyer's patches (46), including small vessels which may be implicated in lymphocyte migration from the vasculature to the Peyer's patches.

The possibility that VIP might act as an immunomodulator is supported by numerous studies examining the interaction of VIP with various lymphocyte populations. Using the MOLT 4B T lymphoblast line, Beed et al. (47) demonstrated specific binding of ^{125}I-VIP to membrane receptors present on these cells. Using Scatchard analysis, they reported that a single class of VIP receptors ($k_D = 7.3$ nmol/l) were present, and estimated 15 000 binding sites per cell based upon a B_{max} of 0.24 nmol/l. In a related study from the same laboratory, human peripheral blood lymphocytes were also assessed for their ability to bind VIP. Normal lymphocytes exhibited higher affinity binding than was observed in the MOLT 4B cell line (48), with fewer binding sites per cell (i.e. $k_D = 0.45$ nmol/l with 1 700 binding sites per cell). As in the previous study, competitive binding data using the related peptides secretin and glucagon demonstrated receptor specificity for VIP.

In assessing differences in VIP binding among murine lymphocyte populations, Ottaway & Greenberg (49) examined populations of cells derived from spleen, mesenteric lymph node, subcutaneous lymph node and Peyer's patches. In all instances, VIP binding was detected, but it appeared to be related to the T cell component of these tissues, since the proliferative responses to the T cell mitogens, PHA and ConA were inhibited, while the response to the B cell mitogen, lipopolysaccharide (LPS) was not.

More recently, VIP binding among cells of B cell lineage has been reported. Using a human pre-B cell line, NALM 6, and the human plasma cell line DAKIKI, O'Dorisio et al. (50) detected high affinity VIP receptors on both cell lines ($k_D = 12.6$ nmol/l and 9.1 nmol/l NALM 6 and DAKIKI respectively). Using covalent cross-linking techniques, they further characterized the receptor as a 47 kD protein.

As mentioned above, VIP down-regulates mitogen-induced lymphocyte proliferation (13, 49, 51). In addition, VIP can inhibit production of interleukin-2 in mitogen stimulated cultures (51). Moreover, treatment of murine T cells with VIP results in down-regulation of their VIP receptors (52). These authors found that in vitro treatment with VIP was associated with a decrease in the ability of T cells to localize in Peyer's patches and mesenteric lymph nodes following subsequent infusion into untreated animals. Thus, VIP appears to regulate movement of lymphocytes from the vasculature to the lymphatic tissue. Similar effects were reported by Moore (53) and Moore et al. (54) using a sheep popliteal lymph node model. In these studies, VIP was infused into the afferent lymphatics in vivo, and the efferent lymphatics were monitored for lymphocyte migration from the node. In both studies a marked decrease in the number of lymphocytes migrating from the node were reported, particularly among T helper ($CD4^+$) cells.

In contrast to these inhibitory actions, VIP has been shown to increase human NK lytic activity in peripheral blood lymphocyte populations (55). Inhibition of lytic activity is observed, however, when VIP is present during the cytotoxic assay.

Many of the effects of in vitro treatment with VIP may be related to VIP activation of adenylate cyclase. In an early study O'Dorisio et al. (56) demonstrated that incubation of several populations of human lymphocytes with VIP resulted in stimulation of adenylate cyclase. They further showed that this reaction was antagonized by somatostatin. Similar results using other human lymphoid populations were obtained by Calvo et al. (57).

More recently, several studies have examined the molecular events following cAMP production produced by initial VIP ligand-receptor interaction. Again using the MOLT 4B lymphoblast, O'Dorisio et al. (58) demonstrated that VIP-stimulated increases in cAMP were followed by activation of cAMP-dependent protein kinase and increased protein phosphorylation. These data suggest a metabolic pathway in which VIP ligand-receptor stimulates adenylate cyclase resulting in activation of cAMP-dependent protein kinase. The exact manner in which these phosphorylated proteins alter cell function has not yet been determined.

With regard to non-lymphoid cells, VIP receptor expression among monocyte/macrophage populations has been somewhat controversial. While early studies of unstimulated human monocytes failed to detect either VIP binding or adenylate cyclase activation (56, 57), more recent studies using populations of zymosan-activated macrophage populations have demonstrated both VIP binding (59), and VIP-induced alterations in metabolic activity (60, 61). Like SP, VIP has chemotactic activity (23), but a detailed study of peptide domains required for binding to the macrophage VIP receptor versus interactions via phagocytic or chemotactic mechanisms has not been performed.

Opioid peptides

Several opioid peptides which appear to modulate immune function have been isolated from the CNS. These include peptides belonging to 2 major groups; the endorphins and the enkephalins. Because of their limited distribution within the CNS and, more importantly, their sparse distribution within the gastrointestinal tract, endorphins will not be specifically included in the present discussion.

In contrast to endorphins (END), enkephalins (ENKs) are widely distributed in the CNS, particularly within the globus pallidus, caudate, amygdala, and hypothalamus (62). In the peripheral nervous system, fibers containing ENK are detected throughout the gastrointestinal tract, with notable localization in the myenteric plexus of the small intestine (2). In the spleen, ENKs have been described in association with the central artery and branches, extending into adjacent white pulp (63).

Enkephalins exist in 2 forms, met-ENK and leu-ENK, named on the basis of their carboxy terminal amino acid. Both pentapeptides are synthesized from a common 236 amino acid precursor, proenkephalin A (64).

Within the central nervous system, enkephalins mediate their function through interactions with δ or μ opioid receptors, with leu-ENK and β END binding to the μ receptor and met-ENK binding primarily to the δ receptor. Met-ENK binding to the μ receptor can occur albeit with lower affinity (2). Similarly, there is evidence that ENK interacts with lymphocyte populations via similar receptors. In an early study, Mehrishi & Mills (65) demonstrated that ^{3}H-naloxone, a specific opioid receptor (μ and δ) blocker, would bind in a specific manner to human peripheral blood lymphocytes. In addition, several studies demonstrating modulation of mitogen-induced proliferation by ENK reported reversal of ENK effects if cells were treated in the presence of naloxone (66–68).

In examining the effects of ENK on the immune response, a number of studies have investigated the effects of in vitro exposure of various lymphocyte populations to mitogen-induced proliferation. In an early study, Plotnikoff & Miller (69) demonstrated that both met- and leu-ENK could augment the proliferative response of murine lymphocytes to PHA. Similar results were obtained by Kharkevich & Kadagidze (67) using met-ENK to modulate PHA responses in human lymphocytes. Treatment of human lymphocytes with met-ENK in vitro can also augment their proliferative response to suboptimal concentrations of ConA, as well as induce proliferation in unstimulated cells (66).

In contrast to these reports of ENK-induced stimulation of proliferative responses, several studies have indicated that in vitro treatment with ENK can result in depressed mitogen induced proliferation. For example, Li & Fraker (68) reported that treatment of murine splenocytes with met-ENK inhibited their response to ConA and LPS, while enhancing the response to PWM. Similarly, Zozulia et al. (70) demonstrated that proliferation of human peripheral blood lymphocytes in response to optimal concentrations of PHA could be inhibited by dalargin, a synthetic analogue of leu-ENK.

While modulation of mitogen responses appears somewhat variable, ENK modulation of NK activity has been more consistent. Faith et al. (71) demonstrated that in vitro treatment of human peripheral blood lymphocytes with either met-ENK or leu-ENK resulted in increased NK activity. Similarly, treatment of mice with varying doses of met-ENK (1–30 mg/kg) in vivo demonstrated increased NK activity in splenocytes recovered within 20 h after treatment. More importantly, this same treatment appeared to enhance survival after viral (HSV-2) infection, and to inhibit growth of transplanted B15 melanoma cells.

In addition to the effects noted above, in vitro treatment of lymphocytes with ENKs results in decreased T cell, and increased B cell and thymocyte motilities (72), stimulation of T cell rosettes (73, 74), and increased IL-2 production (64). Similarly, in vivo treatment of mice or rats with ENK results in decreased antibody production (75), depressed allograft rejection (76), depression of Arthus and delayed type hypersensitivity reactions in mice (76), and decreased lymphocyte release from peripheral lymph nodes (53).

Aside from modulation of lymphocyte activity, modulation of monocyte/macrophage activity may also be influenced by ENK. Metabolic activity of rat macrophages appears to be influenced by met-ENK, with varied responses dependent upon concentration of peptide used (77). Low concentrations (10^{-9}–10^{-7} mol/l) result in influx of Na^+, efflux of Ca^{++}, and a rise in cGMP. This response is inhibited by naloxone. On the other hand, high concentrations (10^{-6}–10^{-5} mol/l) are associated with influx of both Na^+ and Ca^{++}, and increase in cAMP. This response is not inhibited by naloxone.

In addition to its effect on macrophage metabolic activity, met-ENK can alter macrophage function. Using the murine macrophage cell line RAW264, Petty & Berg (78) demonstrated increased phagocytosis of antibody-coated sheep erythrocytes following in vitro treatment with met-ENK. Similar results were also observed following treatment of thioglycolate-stimulated murine peritoneal macrophages. Similar results have been obtained in rats (77, 79). ENK treatment has also been shown to increase IL-1 production (80), enhance reactive oxygen species (81), and enhance non-antibody-mediated tumor cell lysis (82). Cellular reorganization has also been reported in human monocytes following treatment with leu- and met-ENK, with reduction in vimentin filaments and reduction in the expression of MHC class II antigen expression (83).

Neutrophils also appear to be influenced by ENK treatment. Oxygen release is inhibited by both leu- and met-ENK (84). In addition, neutrophil adherence is increased following treatment with met-ENK (85). This effect is only

partially blocked by naloxone, however, suggesting the presence of non-opioid receptors, in addition to more traditional μ opioid receptors.

Calcitonin gene-related peptide

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide. Its name is based upon its relationship to the calcitonin gene complex. This gene complex consists of 6 exons coding 3 major peptides including calcitonin, katacalcin and CGRP. Like many other peptides, CGRP is synthesized as a precursor molecule which is processed by cleavage and amidation prior to release from the cell (86).

Like calcitonin, CGRP is produced by the thyroid (86). In addition, however, it is found throughout the central and peripheral nervous systems. In the CNS it is found in high concentrations in the dorsal horn of the spinal cord, suggesting a sensory function (86). In the gastrointestinal tract it has been localized to a subepithelial plexus in the mucosa of the esophagus. In addition, Stead et al. (87) have reported CGRP-containing neurons in contact with mast cells in the lamina propria of rat intestine. Nerve fibers containing CGRP have also been observed in the spleen (5).

Unlike the above peptides, the immunomodulatory effects of CGRP are only beginning to be described. Binding of CGRP to murine T lymphocytes has been reported (88). On the basis of Scatchard analysis, Umeda & Arisawa (88) have postulated the presence of a high affinity site ($k_D = 3.5 \times 10^{-10}$ mol/l) and a lower affinity site ($k_D = 4.8 \times 10^{-8}$ mol/l), with 265 and 13 000 sites/cell respectively.

CGRP stimulates adenylate cyclase and inhibits PHA and ConA induced proliferation in murine lymphocytes (89). On the other hand, the mitogen response to LPS was unaffected in this study.

CGRP has also been implicated in alteration of macrophage function. Nong et al. (90) have recently reported CGRP-mediated inhibition of peroxide production by macrophages stimulated with interferon-γ. These results are supported by Abello et al. (91), who have demonstrated high affinity binding of CGRP functionally linked to adenylate cyclase in the macrophage-like P388D1 cell line. Eosinophil chemotactic activity is enhanced following treatment with CGRP (92), suggesting that this peptide can also interact with the chemotactic peptide receptor.

Neuropeptide Y

Neuropeptide Y (NPY) is structurally related to pancreatic polypeptide and peptide YY. First isolated from porcine brain as a 36 amino acid peptide, NPY has since been detected in the median eminence and pituitary stalk. In addition it is contained in many neurons throughout the peripheral autonomic nervous system, where it is often colocalized with norepinephrine (2).

In the gastrointestinal tract, NPY may be found in cells within the myenteric plexuses, particularly near both proximal and distal ends. Neurons of the myenteric plexus often contain NPY in association with somatostatin and cholecystokinin (2). In addition, NPY-containing nerve fibers have been identified in the parenchyme of mesenteric lymph nodes, where they end in close proximity to lymphoyctes (5).

NPY has only recently been studied in the context of immunomodulatory effects. In a large study examining the effects of a number of neuropeptides, Söder & Hellström (93) demonstrated that NPY could inhibit the spontaneous proliferative activity of guinea pig lymph node cells. Similar inhibitory effects were noted in mitogen (PHA)-pulsed lymphocytes. In contrast, Jones et al. (94) have recently demonstrated varied effects of NPY on the proliferative responses of human peripheral blood T cells to ConA. Repeated studies over time suggested that the effects of NPY may be altered by the physiological state of the cell donor. Whether or not immune effector cells express high affinity receptors for NPY remains to be determined.

Cholecystokinin

Cholecystokinin (CCK) was first isolated from porcine small intestine and characterized as a 33 amino acid peptide (2). More recently larger forms of the peptide have been identified including 39 and 58 amino acid forms. In addition, a biologically active 8 amino acid form consisting of the carboxyterminal octapeptide of the 33 amino acid form has been isolated. It is predominantly the octapeptide form which has been localized in the small intestine, blood, and CNS.

In the CNS, CCK-8 is found in the cerebral cortex, hypothalamus, and posterior pituitary (2). In the gut it is produced by endocrine cells in the mucosa (I cells), but may also be detected in neurons within the myenteric plexus (2). In the spleen, CCK-8 is found in high abundance in the white pulp where it appears to surround cell clusters (64).

Like NPY and CGRP, the potential immunomodulatory effects of CCK have not yet been thoroughly examined. Freier & Lebenthal (95) have recently reported the effects of CCK-8 infusion on the secretion of antibody into isolated gut loops in the rat. Their results indicated that administration of CCK-8 is followed rapidly by an increase in IgA activity detected in the gut lumen. Similarly, IgG secretion was also enhanced by this treatment, as were IgM and IgE, although to lesser degrees. These results correlated with depressed baseline secretion of IgA and IgG following infusion of the CCK antagonist, proglumide.

Direct cellular effects of CCK have also been reported. McMillen et al. (96) have examined the effects of CCK treatment on mitogen-induced proliferation, as well as intracellular Ca^{++} mobilization. Results of these studies

indicated that like other stimulatory signals such as T cell receptor/ligand interaction, the addition of CCK (10^{-7}–10^{-10} mol/l) to human peripheral blood mononuclear cells stimulates the release of intracellular Ca^{++}. Specificity of this response was confirmed by the failure of CCK to induce a similar response in cells that had been pretreated with the CCK antagonist, L364,718. Similarly, CCK treatment of human peripheral blood mononuclear cells resulted in increased proliferation in cells stimulated with either suboptimal doses of PHA or anti-CD3 antibody. Interestingly, the proliferative response to optimum doses of PHA was inhibited by CCK treatment.

Conclusion

Neuropeptides provide an exquisite mechanism by which the CNS can modulate immune activity. The ability of many of these compounds to specifically bind to both regulatory and effector cells of the immune system has been well documented. Moreover, the facilatory or inhibitory effects of these peptides on immune cells have also been established.

The purpose of the present review has been to examine a subset of neuropeptides which are present within the gastrointestinal tract and may regulate the activities of the endogenous lymphocyte populations. While such regulation may be necessary for the maintenance of normal gut physiology, the precise interactions between the CNS and the immune system at this site remain to be determined.

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REFERENCES

1. McGillis JP, Mitsuhashi M, Payan DG. Immunomodulation by tachykinin neuropeptides. Ann NY Acad Sci 1990; 594: 85–94.
2. McGuigan JE. Hormones of the gastrointestinal tract. In: DeGroot LJ, Besser GM, Cahill GF, et al. eds. Endocrinology. Philadelphia: W.B. Saunders Co 1989: 2741–68.
3. Feher E, Burnstock G. Ultrastructural localisation of substance P, vasoactive intestinal polypeptide, somatostatin and neuropeptide Y immunoreactivity in perivascular nerve plexuses of the gut. Blood Vessels 1986; 23: 125–36.
4. Gates TS, Zimmerman RP, Mantyh CR, et al. Substance P and substance K receptor binding sites in the human gastrointestinal tract: localization by autoradiography. Peptides 1988; 9: 1207–19.
5. Bellinger DL, Lorton D, Romano TD, Olschowka JA, Felten SY, Felten DL. Neuropeptide innervation of lymphoid organs. Ann NY Acad Sci 1990; 594: 17–33.
6. Payan DG, McGillis JP, Organist ML. Binding charcteristics and affinity labeling of protein constituents of the human IM-9 lumphoblast receptor for substance P. J Biol Chem 1986; 261: 14321–9.
7. Payan DG, Brewster DR, Missirian Bastian A, Goetzl EJ. Substance P recognition by subset of human T lymphocytes. J Clin Invest 1984; 74: 1532–9.
8. McGillis JP, Organist ML, Payan DG. Immunoaffinity purification of membrane protein constituents of the IM-9 lymphoblast receptor for substance P. Anal Biochem 1987; 164: 502–13.
9. Payan DG, Brewster DR, Goetzl EJ. Stereospecific receptors for substance P on cultured human IM-9 lymphoblasts. J Immunol 1984; 133: 3260–5.
10. Organist ML, Harvey JP, McGillis JP, Payan DG. Processing of the human IM-9 lymphoblast substance P receptor. Biosynthetic and degradation studies using a monoclonal antibody. Biochem Biophys Res Commun 1988; 151: 535–41.
11. Yokota Y, Sasai Y, Tanaka K, et al. Molecular characterization of a functional cDNA for rat substance P receptor. J Biol Chem 1989; 264: 17649–52.
12. Hershey AD, Krause JE. Molecular characterization of a functional cDNA encoding the rat substance P receptor. Science 1990; 247: 958–62.
13. Scicchitano R, Bienenstock J, Stanisz AM. The differential effect with time of neuropeptides on the proliferative responses of murine Peyer's patch and splenic lymphocytes. Brain Behav Immun 1987; 1: 231–7.
14. Payan DG, Brewster DR, Goetzl EJ. Specific stimulation of human T lymphocytes by substance P. J Immunol 1983; 131: 1613–5.
15. Stanisz AM, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferations by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. J Immunol 1986; 136: 152–6.
16. Wagner F, Fink R, Hart R, Dancygier H. Substance P enhances interferon-gamma production by human blood mononuclear cells. Regul Pept 1987; 19: 355–64.
17. Scicchitano R, Bienenstock J, Stanisz AM. In vivo immunomodulation by the neuropeptide substance P. Immunol 1988; 63: 733–5.
18. Hartung HP, Toyka KV. Activation of macrophages by substance P: induction of oxidative burst and thromboxane release. Eur J Pharmacol 1983; 89: 301–5.
19. Hartung HP, Wolters K, Toyka KV. Substance P: binding properties and studies on cellular responses in guinea pig macrophages. J Immunol 1986; 136: 3856–63.
20. Peck R. Neuropeptides modulating macrophage function. Ann NY Acad Sci 1987; 496: 264–70.
21. Laurenzi MA, Persson MA, Dalsgaard CJ, Haegerstrand A. The neuropeptide substance P stimulates production of interleukin 1 in human blood monocytes: activated cells are preferentially influenced by the neuropeptide. Scand J Immunol 1990; 31: 529–33.
22. Lotz M, Vaughan JH, Carson DA. Effect of neuropeptides on production of inflammatory cytokines by human monocytes. Science 1988; 241: 1218–21.
23. Ruff MR, Wahl SM, Pert CB. Substance P receptor-mediated chemotaxis of human monocytes. Peptides 1985; 6: 107–11.
24. Serra MC, Bazzoni F, Della Bianca V, Greskowiak M, Rossi F. Activation of human neutrophils by substance P. Effect on oxidative metabolism, exocytosis, cytosolic $Ca2^{+}$ concentration and inositol phosphate formation. J Immunol 1988; 141: 2118–24.
25. Hafström I, Gyllenhammer H, Palmblad J, Ringertz B. Substance P activates and modulates neutrophil oxidative metabolism and aggregation. J Rheumatol 1989; 16: 1033–7.
26. Cornaglia Ferraris P, Cornara L, Melodia A. Sensory neuropeptides (substance P) and 4–11 SP enhance human neutrophils chemiluminescence; the role of L-arginine. Int J Immunopharmacol 1986; 8: 463–8.

27. Ali H, Leung KB, Pearce FL, Hayes NA, Foreman JC. Comparsion of the histamine-releasing action of substance P on mast cells and basophils from different species and tissues. Int Arch Allergy Appl Immunol 1986; 79: 413–8.
28. Mannaioni PF, Fantozzi R, Giannella E, Masini E. Pathophysiological significance of the distribution of histamine receptor sub-types: a proposed dual role for histamine in inflammation and type I hypersensitivity reactions. Agents Actions 1988; 24: 26–34.
29. Bar-Shavit Z, Goldman R, Stabinsky Y, et al. Enhancement of phagocytosis—a newly found activity of substance P residing in its n-terminal tetrapeptide sequence. Biochem Biophys Res Commun 1980; 94: 1445–51.
30. Wass JAH. Somatostatin. In: DeGroot LJ, Besser GM, Cahill GF et al. eds. Endocrinology. Philadelphia: W.B. Saunders Co 199: 152–3.
31. Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM. Distribution of somatostatin receptors on murine spleen and Peyer's patch T and B lymphocytes. Brain Behav Immun 1987; 1: 173–84.
32. Bhathena SJ, Louie J, Schechter GP, Redman RS, Wahl L, Recant L. Identification of human mononuclear leukocytes bearing receptors for somatostatin and glucagon. Diabetes 1981; 30: 127–31.
33. Sreedharan SP, Kodama KT, Peterson KE, Goetzl EJ. Distinct subsets of somatostatin receptors on cultured human lymphocytes. J Biol Chem 1989; 264: 949–52.
34. Krco CJ, Gores A, Go VL. Gastrointestinal regulatory peptides modulate in vitro immune reactions of mouse lymphoid cells. Clin Immunol Immunopathol 1986; 39: 308–18.
35. Pawlikowski M, Stepien H, Kunert Radek J, Zelazowski P, Schally AV. Immunomodulatory action of somatostatin. Ann NY Acad Sci 1987; 496: 233–39.
36. Pawlikowski M, Stepien H, Kunert Radek J, Schally AV. Effect of somatostatin on the proliferation of mouse spleen lymphocytes in vitro. Biochem Biophys Res Commun 1985; 129: 52–5.
37. Johansson O, Sandberg G. Effect of the neuropeptides beta-MSH, neurotensin, NPY, PHI, somatostatin and substance P on proliferation of lymphocytes in vitro. Acta Physiol Scand 1989; 137: 107–11.
38. Payan DG, Hess CA, Goetzl EJ. Inhibition by somatostatin of the proliferation of T-lymphocytes and Molt-4 lymphoblasts. Cell Immunol 1984; 84: 433–8.
39. Malec P, Zeman K, Markiewicz K, Tchorzewski H, Nowak Z, Baj Z. Short-term somatostatin infusion affects T lymphocyte responsiveness in humans. Immunopharmacology 1989; 17: 45–9.
40. Vehmeyer K, Schuff Werner P, Nagel GA. Suppressed colony formation of peripheral blood lymphocytes in a patient with somatostatinoma. Clin Immunol Immunopathol 1986; 41: 290–4.
41. Foris G, Gyimesi E, Komaromi I. The mechanism of anitbody-dependent cellular cytotoxicity stimulation by somatostatin in rat peritoneal macrophages. Cell Immunol 1985; 90: 217–25.
42. Cornell RP, McClellan CC. Modulation of hepatic reticuloendothelial system phagocytosis by pancreatic hormones. J Reticuloendothel Soc 1982; 32: 397–407.
43. Goetzl EJ, Payan DG. Inhibition by somatostatin of the release of mediators from human basophils and rat leukemic basophils. J Immunol 1984; 133: 3255–9.
44. Goetzl EJ, Chernov T, Renold F, Payan DG. Neuropeptide regulation of the expression of immediate hypersensitivity. J Immunol 1985; 135: 802–5.
45. Goetzl EJ, Chernov Rogan T, Cooke MP, Renold F, Payan DG. Endogenous somatostatin-like peptides of rat basophilic leukemia cells. J Immunol 1985; 135: 2707–12.
46. Ottaway CA, Lewis DL, Asa SL. Vasoactive intestinal peptide-containing nerves in Peyer's patches. Brain Behav Immun 1987; 1: 148–58.
47. Beed EA, O'Dorisio MS, O'Dorisio TM, Gaginella TS. Demonstration of a functional receptor for vasoactive intestinal polypeptide on Molt 4b T lymphoblasts. Regul Pept 1983; 6: 1–12.
48. Danek A, O'Dorisio MS, O'Dorisio TM, George JM. Specific binding sites for vasoactive intestinal polypeptide on nonadherent peripheral blood lymphocytes. J Immunol 1983; 131: 1173–7.
49. Ottaway CA, Greenberg GR. Interaction of vasoactive intestinal peptide with mouse lymphocytes: specific binding and the modulation of mitogen responses. J Immunol 1984; 132: 417–23.
50. O'Dorisio MS, Shannon BT, Fleshman DJ, Campolito LB. Identification of high affinity receptors for vasoactive intestinal peptide on human lymphocytes of B cell lineage. J Immunol 1989; 142: 3533–6.
51. Ottaway CA. Selective effects of vasoactive intestinal peptide on the mitogenic response of murine T cells. Immunology 1987; 62: 291–7.
52. Ottaway CA. In vitro alteration of receptors for vasoactive intestinal peptide changes the in vivo localization of mouse T cells. J Exp Med 1984; 160: 1054–69.
53. Moore TC. Modification of lymphocyte traffic by vasoactive neurotransmitter substances. Immunology 1984; 52: 511–8.
54. Moore TC, Spruck CH, Said SI. Depression of lymphocyte traffic in sheep by vasoactive intestinal peptide (VIP). Immunology 1988; 64: 475–8.
55. Rola-Leszczynski M, Bolduc D, St Pierre S. The effects of vasoactive intestinal peptide on human natural killer cell function. J Immunol 1985; 135: 2569–73.
56. O'Dorisio MS, Hermina NS, O'Dorisio TM, Balcerzak SP. Vasoactive intestinal polypeptide modulation of lymphocyte adenylate cyclase. J Immunol 1981; 127: 2551–4.
57. Calvo JR, Guerrero JM, Molinero P, Blasco R, Goberna R. Interaction of vasoactive intestinal peptide (VIP) with human peripheral blood lymphocytes: specific binding and cyclic AMP production. Gen Pharmacol 1986; 17: 185–9.
58. O'Dorisio MS, Wood CL, Wenger GD, Vassalo LM. Cyclic AMP-dependent protein kinase in Molt 4b lymphoblasts: identification by photoaffinity labeling and activation in intact cells by vasoactive intestinal polypeptide (VIP) and peptide histidine isoleucine (PHI). J Immunol 1985; 134: 4078–86.
59. Wiik P, Opstad PK, Byum A. Binding of vasoactive intestinal polypeptide (VIP) by human blood monocytes: demonstration of specific binding sites. Regul Pept 1985; 12: 145–53.
60. Wiik P, Haugen AH, Lvhaug D, Byum A, Opstad PK. Effect of VIP on the respiratory burst in human monocytes ex vivo during prolonged strain and energy deficiency. Peptides 1989; 10: 819–23.
61. Wiik P. Vasoactive intestinal peptide inhibits the respiratory burst in human monocytes by a cyclic AMP-mediated mechanism. Regul Pept 1989; 25: 187–97.
62. McIlwain H, Bachelard H. S. Biochemistry and the central nervous system, second edition. New York: Churchill Livingston, 1985: 497–500.
63. Felten DL, Felten SY, Carlson SL, Olschowka JA, Livnat S. Noradrenergic and peptidergic innervation of lymphoid tissue. J Immunol 1985; 135: 755–65.

64. Wybran J, Schandene L, Van Vorren JP, et al. Immunologic patterns of methionine-enkephalin, and therapeutic implications in AIDS, ARC, and cancer. Ann NY Acad Sci 1987; 496: 108–14.
65. Mehrishi JN, Mills IH. Opiate receptors on lymphocytes and platelets in man. Clin Immunol Immunopathol 1983; 27: 240–9.
66. Hucklebridge FH, Hudspith BN, Muhamed J, Lydyard PM, Brostoff J. Methionine-enkephalin stimulates in vitro proliferation of human peripheral lymphocytes via delta-opioid receptors. Brain Behav Immunol 1989; 3: 183–9.
67. Kharkevich DD, Kadagidze ZG. The effect of the stimulation of opioid receptors on lymphocyte functional activity in vivo. Biull Eksp Biol Med 1989; 108: 315–6.
68. Li G, Fraker PJ. Methionine-enkephalin alteration of mitogenic and mixed lymphocyte culture responses in zinc-deficient mice. Chung Kuo Yao Li Hsueh Pao 1989; 10: 216–21.
69. Plontnikoff NP, Miller GC. Enkephalins as immunomodulators. Int J Immunopharmacol 1983; 5: 437–41.
70. Zonzulia AA, Voronkova TL, Patsakova E, Ivanushkin AM. Importance of opiate receptor ligands in regulating lymphocyte proliferative activity. Farmakol Toksikol 1987; 50: 58–9.
71. Faith RE, Liang HJ, Plotnikoff NP, Murgo AJ, Nimeh NF. Neuroimmunomodulation with enkephalins: in vitro enhancement of natural killer cell activity in peripheral blood lymphocytes from cancer patients. Natl Immun Cell Growth Regul 1987; 6: 88–98.
72. Ye S, Applegren RR, Davis JM, Cheung HT. Modulation of lymphocyte motility by beta-endorphin and met-enkephalin. Immunopharmacology 1989; 17: 81 9.
73. Plotnikoff NP, Murgo AJ, Miller GC, Corder CN, Faith RE. Enkephalins: immunomodulators. Fed Proc 1985; 44: 118–22.
74. De Carolis C, De Sanctis G, Perricone R, et al. Evidence for an inhibitory role of beta-endorphin and other opioids on human total T rosette formation. Experientia 1984; 40: 738–9.
75. Jankovic BD, Maric D. Enkephalins and immunity I: in vivo suppression and potentiation of humoral immune response. Ann NY Acad Sci 1987; 496: 115–25.
76. Maric D, Jankovic BD. Enkephalins and immunity. II: In vivo modulation of cell-mediated immunity. Ann NY Acad Sci 1987; 496: 126–36.
77. Foris G, Medgyesi GA, Hauck M. Bidirectional effect of met-enkephalin on macrophage effector functions. Mol Cell Biochem 1986; 69: 127–37.
78. Petty HR, Berg KA. Combinative ligand-receptor interactions: epinephrine depresses RAW264 macrophage antibody-dependent phagocytosis in the absence and presence of met-enkephalin. J Cell Physiol 1988; 134: 281–6.
79. Foris G, Medgyesi GA, Gyimesi E, Hauck M. Met-enkephalin induced alterations of macrophage functions. Mol Immunol 1984; 21: 747–50.
80. Yang SX, Li XY. Enhancement of interleukin-1 production in mouse peritoneal macrophages by methionine-enkephalin. Chung Kuo Yao Li Hsueh Pao 1989; 10: 266–70.
81. Willems J, Leclercq G, Joniau M. Enhanced oxygen metabolism of peritoneal macrophages in the presence of murine neuroblastoma cells is partly caused by enkephalins. J Neuroimmunol 1988; 19: 269–78.
82. Cameron DJ. Effect of neuropeptides on macrophage mediated cytotoxicity in normal donors and cancer patients. Jpn J Exp Med 1987; 57: 31–9.
83. Prieto J, Subira ML, Castilla A, Arroyo JL, Serrano M. Opioid peptides modulate the organization of vimentin filaments, phagocytic activity, and expression of surface molecules in monocytes. Scand J Immunol 1989; 29: 391–8.
84. Simpkins CO, Alailima ST, Tate EA, Johnson M. The effect of enkephalins and prostaglandins on O-2 release by neutrophils. J Surg Res 1986; 41: 645–52.
85. van Epps DE, Kutvirt SL. Modulation of human neutrophil adherence by beta-endorphin and met-enkephalin. J Neuroimmunol 1987; 15: 219–28.
86. Macintyre I. Calcitonin: physiology, biosynthesis, secretion, metabolism, and mode of action. In: DeGroot LJ, Besser GM, Cahill GF, et al. eds. Endocrinology. Philadelphia: W.B. Saunders Co 1989: 892–901.
87. Stead RH, Tomioka M, Quinonez G, Simon GT, Felten SY, Bienenstock J. Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. Proc Natl Acad Sci USA 1987; 84: 2975–9.
88. Umeda Y, Arisawa M. Characterization of the calcitonin gene-related peptide receptor in mouse T lymphocytes. Neuropeptides 1989; 14: 237-42.
89. Umeda Y, Takamiya M, Yoshizaki H, Arisawa M. Inhibition of mitogen-stimulated T lymphocyte proliferation by calcitonin gene-related peptide. Biochem Bophys Res Commun 1988; 154: 227–35.
90. Nong YH, Titus RG, Ribeiro JM, Remold HG. Peptides encoded by the calcitonin gene inhibit macrophage function. J Immunol 1989; 143: 45–9.
91. Abello J, Kaiserlian-Nicolas D, Cuber JC, Revillard JP, Chayvialle JA. Identification of high affinity calcitonin gene-related peptide receptors on a murine macrophage-like cell line. Ann NY Acad Sci 1990; 594: 364–6.
92. Manley HC, Haynes LW. Eosinophil chemotactic response to rat CGRP-1 is increased after exposure to trypsin or guinea-pig lung particulate fraction. Neuropeptides 1989; 13: 29–34.
93. Söder O, Hellström PM. Neuropeptide regulation of human thymocyte, guinea pig T lymphocyte and rat B lymphocyte mitogenesis. Int Arch Allergy Appl Immunol 1987; 84: 205–11.
94. Jones JV, James H, Mansour M. Neuropeptide Y modulates t cell proliferation in vitro: A new interaction of the immune and neuroendocrine systems. Ann NY Acad Sci 1990; 594: 388–90.
95. Freier S, Lebenthal E. The neuroendocrine-immune network in the gastrointestinal tract. In: Freier S, ed. The neuroendocrine-immune network. Boca Raton: CRC Press Inc 1990: 239–55.
96. McMillen MA, Ferrara A, Schaefer HC, Goldenring JR, Zucker KA, Modlin IM. Cholecystokinin mediates a calcium signal in human peripheral blood mononuclear cells and is a co-mitogen. Ann NY Acad Sci 1990; 594: 399–402.