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INVITED REVIEW

The intriguing mission of neuropeptide Y in the immune system

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Abstract For many years, the central nervous system and the immune system were considered two autonomous entities. However, extensive research in the field of neuroimmunomodulation during the past decades has demonstrated the presence of different neuropeptides and their respective receptors in the immune cells. More importantly, it has provided evidence for the direct effects of neuropeptides on the immune cell functions. Neuropeptide Y (NPY) is generally considered the most abundant peptide in the central and peripheral nervous system. However, it is also distinguished by exhibiting pleiotropic functions in many other physiological systems, including the immune system. NPY affects the functions of the cells of the adaptive and innate immunity. In this respect, NPY is known to modulate immune cell trafficking, T helper cell differentiation, cytokine secretion, natural killer cell activity, phagocytosis and the production of reactive oxygen species. The specific Y receptors have been found in immune cells, and their expression is amplified upon immune stimulation. Different Y receptor subtypes may mediate an opposite effect of NPY on the particular function, thus underlining its regulatory role. Since the immune cells are capable of producing NPY upon appropriate stimulation, this peptide can regulate immune cell functions in an autocrine/paracrine manner. NPY also has important implications in several immune-mediated disorders, which affirms the clear need for further investigation of its role in either the mechanisms of the disease development or its possible therapeutic capacity. This review summarises the key points of NPY's mission throughout the immune system.

Keywords Neuropeptide Y · Y receptors · Immune cells · Infection · Inflammation · Autoimmunity

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Introduction

Neuropeptide Y (NPY) is a 36-amino acid C-terminally amidated neurotransmitter peptide with relatively high tyrosine content in the molecule. Thus, it is designated as a peptide with a capital Y. NPY, together with its natural homologues such as peptide YY (PYY) and pancreatic polypeptide (PP) and their truncated forms NPY2-36, NPY3-36 and PYY3-36, belongs to a family of peptides with a unique amino acid backbone that forms a hairpin turn called the PP-fold. This means that the N-terminal part of the molecule (residues 1–8) adopts a polyproline type II helical conformation, and residues 9–13 form a loop that enables the polyproline helix to fold back onto an α-helix, encompassing residues 14–31 (Blundell et al. 1981).

NPY is cleaved from its precursor prepro-NPY (97 and 98 amino acid residues in humans and rats, respectively) by combined and successive actions of signal peptidase (Higuchi et al. 1988), prohormone convertases PC1/3 and PC2, and carboxypeptidase E (Fricker 1988). Novel findings show that cathepsin L participates as a key proteolytic enzyme for NPY production in secretory vesicles, generating peptide intermediates with basic residue extensions at N- and C-termini, which will then be removed by aminopeptidase B and carboxypeptidase E, respectively (Funkelstein et al. 2008). Finally, the molecule is amidated

by peptidylglycine α-amidating mono-oxygenase at the C-terminal end (Bradbury et al. 1982) to form biologically active NPY.

NPY is one of the most evolutionary conserved peptides among species. Namely, only two of 36 amino acids of NPY are variable in mammals and only three differ from shark NPY. In both humans and rats, average NPY plasma concentrations were found to be in the picomolar range (Grouzmann et al. 1989, 2001; Reich et al. 2007; Thompson et al. 1995) with a half-life of approximately 12 min. It should be noted, however, that in rats, platelet release could be a major source of circulating peptides in this species (Myers et al. 1988).

In mammals, members of the NPY peptide family bind to several different Y receptor subtypes (Y1, Y2, Y3, Y4, Y5 and y6) (Berglund et al. 2003; Michel et al. 1998). All Y receptors have been cloned, except Y3, while y6 is functional only in a few species. Y receptors have all been shown to couple to inhibitory G-proteins and thus mediate the inhibition of cAMP synthesis. Additional mechanisms of cell signalling involve phospholipase C activation and mobilisation of Ca^{2+} from intracellular stores (Herzog et al. 1992; Mullins et al. 2002). Different Y receptor subtypes show extreme structural heterogeneity (Larhammar et al. 2001) and diverse affinity for NPY and related peptides (Michel et al. 1998). It is noteworthy that N-terminally truncated derivatives of the NPY and PYY (NPY2-36, NPY3-36 and PYY3-36), as a consequence of degradation by endogenous enzymes (Gorrell 2005; Mentlein and Roos 1996; Mentlein 1999), lose their efficacy at the Y1 receptor but remain active, especially toward the Y2 receptor. Dipeptidyl peptidase 4 (DP4, CD26), belonging to a serine protease enzyme, is the primary peptidase involved in the N-terminal truncation of the NPY and PYY, leading to the formation of NPY3–36 and PYY3–36 (Mentlein 1999; Gorrell 2005). It is well known that most DP4/CD26 activity is membrane expressed, mainly located on the vasculature/endothelial cells and on hepatocytes. However, there is also a strong circulating DP4-like activity. Aminopeptidase P from smooth muscle cells removes the terminal amino acid from the NPY, therefore generating NPY 2–36 (Mentlein and Roos 1996).

Following the isolation of NPY from the porcine brain (Tatemoto et al. 1982) and the finding that NPY is a peptide with the highest distribution in the central nervous system, intensive research of its role in central nervous system functioning commenced. This resulted in numerous comprehensive appraisals. It is now known that NPY is involved in the complex network within the hypothalamus. By projecting from an arcuate nucleus toward other hypothalamic areas and receiving reciprocal inputs from the brainstem and cortical areas and reward pathways, it regulates food intake and energy balance (Simpson et al. 2009). NPY neurons are also involved in regulating energy metabolism. They can communicate with the hypothalamic-pituitary–gonadal axis and can operate as neuroendocrine integrators, linking perturbations in energy balance and alterations in the activity of the reproductive axis (Hill et al. 2008). In the hippocampus, NPY is mainly produced and released by inhibitory interneurons that hinder glutamatergic neurotransmission in the excitatory circuits. They consequently restrain the spread of excitability into other brain structures (Xapelli et al. 2006). Decreased expression of NPY in the central amygdala may be one of the common molecular mechanisms for the comorbidity of anxiety and alcoholism (Pandey et al. 2005), while the abundant expression of NPY and/or its receptors in the brain regions is associated with stress response, mood disorders and sleep regulation. This also points to the involvement of NPY in these activities (Morales-Medina et al. 2010; Dyzma et al. 2010).

In the periphery, NPY has a potent mitogenic activity and is chemotactic for vascular smooth muscle cells and endothelial cells, and also stimulates angiogenesis (Zukowska-Grojec et al. 1998). It is also a strong vasoconstrictor and cardio-depressant (Zukowska-Grojec and Vaz 1988). NPY is co-localised and co-released with norepinephrine from nerve fibres upon stimulation, and it modulates the effects of norepinephrine at both high and low levels of sympathetic nerve activity (Han et al. 1998). Moreover, NPY originating from the adrenal medulla locally enhances the secretion of catecholamines (Cavadas et al. 2001). Similarly to PYY in the gut, NPY is a potent inhibitor of intestinal fluid secretion (Souli et al. 1997).

NPY fulfills the classical criterion set for nerve-immune transmitter, as proposed by Bedoui et al. (2003a). First, NPY is synthesised in postganglionic nerves innervating the lymphoid organs and is released upon neuronal stimulation (Romano et al. 1991). Second, the close proximity between NPY-positive nerve fibres and immunocompetent cells has been demonstrated in the spleen (Meltzer et al. 1997) and in inflamed mucosa. This results in a direct effect of nerve-derived NPY on the immune cells. Finally, the expression of specific Y receptors in the immune cells and the ability of different Y receptor antagonists to prevent NPY-induced immune perturbation have been shown in numerous studies. In turn, immune stimulation can change the NPY content in the nervous system. Systemic immune stimulation with lipopolysaccharide (LPS) decreases the expression of NPY in the rat hypothalamus (Kim et al. 2007), whereas experimental sepsis increases it (Carlson et al. 2009). Therefore, endogenous NPY might be considered a regulator of both nervous and immune system functions.

NPY and the immune system

Y receptors on the cells of the immune system

The presence of Y receptors in immune cells has been verified at the molecular level (at both transcription and translation) via identifying receptor mRNA by PCR. In humans and rodents, Y1 receptor mRNA has been detected in every immune cell type examined so far (Table 1). In addition, the Y1 receptor subtype is the first Y receptor cloned from rat splenocytes (Petitto et al. 1994). Although the Y1 receptor from lymphocytes has been found to have an identical nucleotide sequence as the Y1 receptor in the brain, the level of its expression in the spleen was significantly lower compared to the expression level in the brain. Differences in the levels of Y1 receptor mRNA detected have been noticed in various immune cell types (Wheway et al. 2005), whereas increased levels were detected upon immunisation or inflammatory reaction (Bedoui et al. 2003b; Rethnam et al. 2010). Y2 receptor mRNA transcription in inflammatory cells can be induced by in vitro stimulation with LPS (Nave et al. 2004) or *N*-formyl-methionine-leucine-phenylalanine (fMLP) (Bedoui et al. 2008). Human neutrophils contain mRNA encoding all cloned receptor subtypes such as Y1, Y2, Y4 and Y5, with the Y4 receptor being dominant in comparison with the other three subtypes (Bedoui et al. 2008). Additionally, immunolabeling with specific antibodies revealed Y1, Y2 and Y5 membrane receptors expression on rat inflammatory granulocytes (Dimitrijević et al. 2010; Mitić et al. 2011). Furthermore, Y receptors have been detected on a subpopulation of granulocytes, ranging from 5 to 20% of HIS48+ cells, depending on the inflammatory agent used for their activation, for example, LPS or carrageenan. The occasional Y receptor-positive granulocytes have been detected in the rat peripheral blood by immunocytochemical staining (Mitić et al. 2011). However, further studies are needed to confirm the presence of Y receptors on immune cells in mice where the use of Y1 receptor-deficient (Y1−/Y1−) mice can be of great help as a negative control for the specificity of Y receptor antibodies used for immunolabeling. Another important issue is to test whether different Y receptor subtypes are expressed on a single cell, and if so, to examine the implication of their co-expression on the cell functioning. Nevertheless, pharmacological manipulations with receptor-specific agents have proven the functional capacity of the expressed Y receptors in immune cells. The most commonly used agonists specific for different Y receptors are shown in Fig. 1.

The effects of NPY on the cells of the immune system

Lymphocytes

Sympathetic innervation of lymphoid organs (Muller and Weihe 1991; Romano et al. 1991; Stevens-Felten and Bellinger 1997) and mucosal-associated lymphoid tissues (Nohr and Weihe 1991; Sipos et al. 2006) enables the close contact between nerves and lymphocytes and macrophages, thus also exposing the cells to NPY. These findings suggest the involvement of NPY in immune cell recruitment. Indeed, NPY has been shown to augment the $\beta 1$ integrin-mediated adhesion of resting human T cells to fibronectin, a major glycoprotein component of the extracellular matrix (Levite et al. 1998). Although NPY did not alter the expression of $\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrins, it might facilitate

Table 1 The Y receptors on immune cells

Receptor	Cell type	Tissue	References
Rat			
Y1	Lymphocytes	Spleen	Petitto et al. (1994)
Y1	Monocytes	Peripheral blood cells	Bedoui et al. (2002)
Y2		(LPS-induced)	Nave et al. (2004)
Y1,2,5	Granulocytes	Air-pouch inflammation	Dimitrijević et al. (2010), Mitić et al. (2011)
Mice			
Y1	Lymphocytes	Lymph nodes from immunised mice	Bedoui et al. (2003b)
Y1	T cells	Spleen	
Y1	Dendritic cells, NK cells, T cells, B cells, macrophages	Spleen	Wheway et al. (2005)
Human			
Y1,4,5	Neutrophils	Peripheral blood cells	Bedoui et al. (2008)
Y2		(fMLP-induced)	
Y1	T cells	Inflamed gingiva	Rethnam et al. (2010)
Y1	Granulocytes		

fMLP *N*-formyl-methionine-leucine-phenylalanine, *LPS* lipopolysaccharide

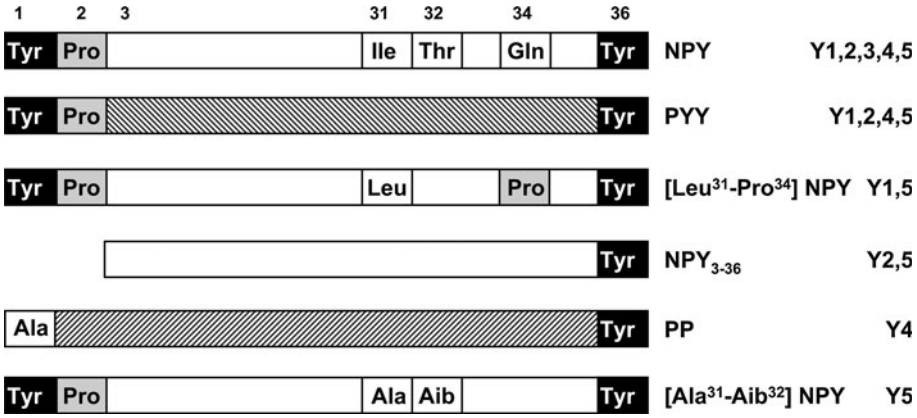

Fig. 1 The agonists most commonly used for the testing of functional capacity of Y receptors expressed in the immune and inflammatory cells. Note the striking structural similarities among different Y receptor agonists, most of them having tyrosine on both the C- and N-terminal end. Substitution of the two amino acids in the NPY molecule is sufficient to modify the specificity for a certain Y receptor subtype. Specific Y receptor antagonists such as BIBO 3304 (Y1), PD160170 (Y1), BIIE 0246 (Y2), and L 152804 (Y5) also supported the functionality of the Y receptors in the immune cells (Bedoui et al. 2008; Dimitrijević et al. 2005, 2006, 2008, 2010; Nave et al. 2004; Mitić et al. 2011; Zhou et al. 2008)

the extravasation of T cells by increasing their affinity to fibronectin. More importantly, the proadhesive action of NPY was mediated in a receptor-specific manner, particularly via the Y2 receptor. NPY in vitro at both physiological and pharmacological concentrations (ranging from 10^{-12} to 10^{-8} M) enhanced the fMLP-stimulated migration of lymphocytes in mice (De la Fuente et al. 1993). Conversely, a rather high concentration of NPY in vitro was ineffective in stimulating the migration of human T cells into a collagen matrix (Talme et al. 2008). It has been reported that cell migration on the two-dimensional test systems, for example plastic, essentially differs from the migration to three-dimensional structures such as collagen matrices. While both test systems measure cell migration, only migration and penetration to collagen reflects the cells' ability to infiltrate certain tissues (Talme et al. 2008). Thus, NPY appears to be capable of intensifying the recruitment of lymphocytes by affecting both the chemotaxis and adhesiveness of these cells. However, it does not stimulate their capacity to penetrate the tissue, emphasising the importance of the tissue microenvironment in shaping recruitment.

Numerous studies have shown the suppressive effect of NPY (10^{-12}–10^{-8} M) in vitro on lymphocyte proliferation following stimulation with mitogens such as concanavalin A and phytohemagglutinin (Soder and Hellstrom 1987; Medina et al. 1999, 2000a; Puerto et al. 2005), or anti-CD3 antibody (Wheway et al. 2007). Reduced proliferative capacity of mitogen-stimulated lymphocytes following NPY treatment in vitro is probably a consequence of decreased IL-2 production (Puerto et al. 2005). Moreover, the inability of NPY to suppress lymphocyte proliferation in aged mice was accompanied by its inability to diminish IL-2 production, which also declines with age. Concanavalin A weakly stimulated lymphocytes from mature and old animals to proliferate and produce IL-2, but strongly affected cells from young, and to a lesser extent, from adult mice (Medina et al. 1999, 2000a; Puerto et al. 2005). Thus, the level of cellular activation interferes with the effect of NPY on lymphocyte proliferation. However, NPY did not suppress the proliferation of unstimulated mouse lymphocytes (Medina et al. 1999).

The physiological implications of NPY-induced suppression of T lymphocyte proliferation are probably to restrain redundant immune response. It is noteworthy that the proliferative capacity of anti-CD3 antibody-stimulated lymphocytes was only suppressed by the nanomolar concentration of NPY if it was delivered at the time of cell stimulation. However, it had no influence on already activated cells, thus suggesting that this peptide interferes with the early phases of T cell priming (Wheway et al. 2007).

Conversely, NPY (10^{-12}–10^{-6} M) indirectly enhanced the mitogen-stimulated proliferation of human colonic lamina propria lymphocyte by increasing the production of IL-1β in monocytes (Elitsur et al. 1994; Hernanz et al. 1996). The ability of NPY at a physiological concentration to augment gut lymphocyte proliferative capacity has been evident following the stimulation of cells with either phorbol esther, which activates membrane protein kinase C (Nishizuka 1984), or concanavalin A, which activates membrane Toll-like receptors 2 and 6 (Unitt and Hornigold 2011). Considering that gut-associated lymphoid tissue is the first line of defense against intestinal bacteria, the stimulative effect of NPY on mucosal lymphocytes probably facilitates a prompt anti-bacterial response.

The modulation of cytokine production, especially those related to a shift toward Th1 or Th2 immune response, is certainly the most significant action of NPY on immune cell functions. NPY greatly enhanced IL-4 production and inhibited IFN-γ in mouse splenocytes upon stimulation with an anti-CD3 antibody and in mouse helper T cell clones stimulated with an antigen in vitro (Kawamura et al. 1998), thus supporting Th2 immune response. NPY (10^{-8} M) has also been shown to induce the secretion of cytokines from antigen-specific Th1 and Th2 cell lines without additional antigen stimulation (Levite 1998, 2000). Unexpectedly, NPY stimulated the secretion of both Th1 (IL-2 and IFN-γ) and Th2 (IL-4) cytokines from the Th1 T cell clone. NPY also directly stimulated a Th2 T cell clone to secrete IFN-γ.

Regarding cytokine secretion following antigen stimulation of T cell clones, it has been shown that NPY in vitro is capable of breaking the commitment of single Th1 and Th2 cells already engaged in a distinct pattern of cytokine secretion (Levite 1998, 2000). Specifically, NPY (10^{-8} M) increased IL-4, IL-2 and IFN-γ, in the antigen-stimulated Th1 clone and Th2 clone, respectively. Taken as a whole, NPY could play a substantial role in the regulation of the adaptive immune response by modulating T cell migration, proliferation and cytokine secretion. Despite the proven existence of mRNA for the Y1 receptor in T cells and B cells (Bedoui et al. 2003b; Petitto et al. 1994; Wheway et al. 2005), the potential role of other Y receptor subtypes, as well as their interaction with the Y1 receptor in the NPY-induced modulation of lymphocyte functions, has not been investigated so far.

NK cells

NK cells survey host tissues for signs of infection, transformation or stress and kill target cells that have become useless or are detrimental to the host. Evidence has been provided for the immunosuppressive effects of both exogenous and endogenous NPY on NK cell activity. In particular, NPY in vitro (10^{-12}–10^{-9} M) significantly suppressed the NK activity of human peripheral blood lymphocytes (Nair et al. 1993); intravenous NPY administration produced a dose-dependent inhibition of rat splenic NK activity (Saurer et al. 2006); and stress-induced elevation of plasma levels of NPY has been associated with the suppression of NK cell activity (Irwin et al. 1992). Considering the major input of NK cells in the MHC-unrestricted recognition of virally infected cells and tumours, the NPY-induced suppression of NK activity under stress may increase susceptibility to viral infections and cancer. However, comprehensive analysis of the modulatory capacity of NPY on NK activity revealed a lymphoid tissue compartment- and age-dependent pattern of alterations (De la Fuente et al. 2001b; Puerto et al. 2005). Specifically, NPY in vitro increased and decreased NK activity of peritoneal leukocytes from adult and aged rats, respectively.

Considering that basal NK activity declines with age, NPY might be one of the mediators that, in addition to catecholamines, diminishes NK activity due to heightened sympathetic activity, or expands catecholamine-induced NK suppression in the aged. The opposing effects of NPY on NK activity of cells originating from different lymphoid organs may be a consequence of different functional subsets of NK cells. In humans, the more mature CD56dim population is potently cytotoxic, whereas CD56bright NK cells have low cytotoxicity, produce much greater amounts of cytokines and express homing molecules for the secondary lymphoid organs and sites of inflammation (Cooper et al. 2001). Identification of NK cell subsets in mice revealed more reactive CD27+ NK cells in terms of natural cytotoxicity, cytokine production and proliferation (Hayakawa and Smyth 2006). Further studies should address the complex issue regarding the regulatory role of NPY on different subpopulations of NK cells.

Monocytes and macrophages

NPY has been shown to modulate the recruitment of monocytes and macrophages by acting as a chemoattractant itself at a physiological concentration (10^{-11} M) (Straub et al. 2000) and by increasing their adherence capacity at both physiological and pharmacological concentrations (De la Fuente et al. 2000; Medina et al. 2000b; Nave et al. 2004). It has been suggested that NPY can reduce tissue immigration of monocytes under endotoxemic conditions by increasing their adhesiveness (Nave et al. 2004). The proadhesive effect of NPY in vitro on LPS-stimulated rat peritoneal macrophages was mediated via Y2 receptors (Nave et al. 2004), as it has been previously demonstrated for lymphocytes (Levite et al. 1998). The involvement of Y1 receptors in the NPY-induced potentiation of adhesion of rat peripheral blood monocytes stimulated with LPS has been recently demonstrated (Mitić et al. 2011). NPY at the micromolar concentration increased the adherence capacity of thioglycollate-elicited rat peritoneal macrophages without the additional in vitro activation of cells with LPS (Stanojević et al. 2007).

Although NPY undoubtedly exhibited a proadhesive effect on monocytes and macrophages, it was ineffective in aged mice that already exhibited an increased adherence capacity (De la Fuente et al. 2000; Medina et al. 2000b). Likewise, the pharmacological concentration of PYY of 10^{-6} M stimulated the macrophage adherence capacity in adult rats, but had no influence in aged rats (Stanojević et al. 2006). The absence of the proadhesive effect of NPY

on inflammatory cells is beneficial in advanced age, as it can restrain the inflammatory reaction. This might also account for the stress situation, as the previous exposure of rats to stress prevented the in vitro proadhesive influence of NPY on inflammatory macrophages (Stanojević et al. 2007).

In addition to the significant impact of NPY in vitro on leukocytes chemotaxis and adhesion, NPY in vivo evidently modulates the trafficking of peripheral blood monocytes in the rat (Bedoui et al. 2001). In particular, a high dose of intravenous NPY increased blood leukocyte numbers in rats via the mobilisation of different cell subsets, including CD9+ monocytes. However, the stimulating effects of NPY on leukocytes mobilisation were mediated via the Y5 receptor on non-immune cells (Bedoui et al. 2002).

Several studies showed that NPY also stimulated cytokine secretion from monocytes and macrophages. Specifically, NPY (10^{-10} M) increased the secretion of IL-1β in human peripheral blood monocytes (Hernanz et al. 1996) and mouse peritoneal macrophages (De la Fuente et al. 2001a; Puerto et al. 2005) following the in vitro activation of cells with concanavalin A. The findings show that NPY decreased macrophage production of the proinflammatory cytokine TNF-α following stimulation with LPS (Puerto et al. 2005). It also increased the production of anti-inflammatory cytokine TGF-β1 in macrophage cell line Raw 264.7 (Zhou et al. 2008), thus revealing NPY's ability to diminish inflammatory reaction.

NPY has a significant impact on macrophage effector functions, especially by modulating the phagocytosis of foreign particles and the production of hydrogen peroxide and nitric oxide. NPY (10^{-12}–10^{-8} M) stimulated the phagocytosis of latex beads in resident peritoneal macrophages derived from mice (De la Fuente et al. 1993, 2000, 2001a). At a similar concentration range (10^{-12}–10^{-10} M), it also suppressed the phagocytosis of zymosan in resident and thioglycollate-elicited peritoneal macrophages from the rat via Y2 and Y5 receptors (Dimitrijević et al. 2005; Stanojević et al. 2007). The observed differences are probably due to the nature of stimuli used in these studies. The phagocytosis of zymosan is an inflammatory event mediated by NFκB activation (Friedland et al. 2001). In macrophages, zymosan-induced responses include the induction of proinflammatory cytokines, arachidonate mobilisation, protein phosphorylation, and inositol phosphate formation. The phagocytosis of zymosan is mediated via its binding to complement receptor CR3 (also known as Mac-1 or CD11b/CD18) and Toll-like receptor 2 (Burton et al. 2010). Latex beads are immunologically inert particles phagocytosed via scavenger receptors (Kobzik 1995). However, in comparison with zymosan, they induce a significantly lower production of proinflammatory cytokines such as IL-8 (Friedland et al. 2001).

NPY also suppressed the phagocytic and leishmanicidal capacities of macrophage cell line Raw 264.7, but at higher doses of the peptide (10^{-10}–10^{-5} M, Ahmed et al. 2001). It is interesting that NPY potentiated an oxidative burst initiated by either zymosan (Mitić et al. 2011) or phorbol myristate acetate, which involves the non-receptor direct activation of membrane protein kinase C (Dimitrijević et al. 2005; Stanojević et al. 2007). The former was mediated by Y1 receptor on rat peripheral blood monocytes (Mitić et al. 2011) and the latter by macrophage Y1 and Y2 receptors (Dimitrijević et al. 2005). The finding that a specific agonist of the Y5 receptor significantly suppressed oxidative burst in phorbol myristate acetate-stimulated macrophages indicated the Y1/Y2 and Y5 receptor interplay in the modulation of macrophage hydrogen peroxide production. In addition, NPY increased nitric oxide production by the LPS-activated resident peritoneal macrophages, and its stimulatory effect was also mediated via Y1 and Y2 receptors (Dimitrijević et al. 2008). Although the NPY-induced suppression of phagocytosis and the potentiation of reactive oxygen species production may be considered anti-inflammatory and proinflammatory actions, respectively NPY generally seems to assist in the elimination of pathogens and/or transformed cells by supporting the mechanisms for their destruction.

Granulocytes

The ability of NPY in vitro to modulate granulocyte functions largely depends on the NPY concentration, cell activation and species. NPY significantly increased and decreased phagocytosis of *Escherichia coli* by human peripheral blood granulocytes at physiological (10^{-12} M) and pharmacological concentrations (10^{-6}–10^{-5} M), respectively (Bedoui et al. 2008). However, in rats, NPY decreased (10^{-8} M) the phagocytosis of zymosan by granulocytes isolated from a sterile inflammation site, for example, carrageenan-elicited cells from the air-pouch (Dimitrijević et al. 2006), and increased (10^{-6} M) phagocytosis of zymosan by granulocytes isolated from peripheral blood (Mitić et al. 2011). The NPY-induced modulation of phagocytic capacity of rat granulocytes was mediated via Y1 receptors (Dimitrijević et al. 2006; Mitić et al. 2011). In addition to the Y1 receptor, Y2 receptors also mediated suppression of the phagocytic capacity of human granulocytes by NPY in vitro (Bedoui et al. 2008). The pharmacological concentration of NPY (10^{-8}–10^{-5} M) significantly intensified the fMLP-stimulated respiratory burst in human granulocytes, and this effect was mediated by the Y5 receptor (Bedoui et al. 2008).

In contrast to the above, pharmacological doses of NPY in vitro induced a considerable decrease in rat granulocyte peroxide production, mediated by activation of the Y2 and Y5 receptors (Dimitrijević et al. 2006). NPY increases LPS-stimulated nitric oxide production in rat granulocytes via the Y1 receptor (Dimitrijević et al. 2006). Besides, this recent study demonstrated the involvement of Y1 receptors in the NPY-induced potentiation of the adhesion of rat peripheral blood granulocytes stimulated with LPS (Mitić et al. 2011). In summary, the stimulatory effects of NPY on granulocyte functions are regularly mediated via Y1 receptors, whereas the suppressive effects of NPY utilise different Y receptor subtypes. Depending on the peptide concentration and specific Y receptor subtype(s), NPY can direct human granulocytes activity towards better phagocytosis or improved killing ability, but in both circumstances, it facilitates the elimination of pathogens. The contribution of different Y receptor subtypes in the modulation of immune cells functions by NPY, as revealed by the in vitro studies with the Y specific agonists and antagonist, is summarised in Table 2.

The production of NPY by the cells of the immune system

Chemical sympathectomy did not change the spleen content of NPY (Lundberg et al. 1985), and denervation retained high levels of NPY expression in leukocytes isolated from the renal grafts (Holler et al. 2008). This is suggestive of NPY production by some immune cells independently from neurons. Furthermore, the presence of both NPY mRNA and NPY peptide was detected in rat and mouse spleen, bone marrow, and peripheral blood cells, the amount of NPY mRNA being in the range of those found in human and rat nervous tissue (Lundberg et al. 1983; Beck et al. 1993). However, genes controlling the production of NPY and related peptides are inducible, rather than constitutively expressed in the immune system (Schwarz et al. 1994). Human lymphocytes produce NPY only after additional in vitro cell activation (Bracci-Laudiero et al. 1996a) or in chronic inflammation (Sipos et al. 2006). Conversely, strong NPY mRNA and NPY peptide expression was seen in resting peripheral blood leukocytes in the rat (Holler et al. 2008). Nevertheless, immune cells

Table 2 The participation of Y receptors in the modulation of immune cells functions

Effect	Cell (species)	References
Y1 receptor		
↑Adherence capacity	Air-pouch granulocytes (r)	Dimitrijević et al. (2010)
	Monocytes and granulocytes (r)	Mitić et al. (2011)
↓Adherence capacity	Macrophages (r)	Stanojević et al. (2006)
↑TGF-β1 production	Raw 264.7 cells	Zhou et al. (2008)
↓IFN-γ production	Splenocytes (m)	Bedoui et al. 2003b
↑Phagocytosis	Macrophages (r)	Dimitrijević et al. (2005)
	Monocytes and granulocytes (r)	Mitić et al. (2011)
↓Phagocytosis and ↑ROS production	Air-pouch granulocytes (r) and granulocytes (h)	Dimitrijević et al. (2006, 2010) Bedoui et al. (2008)
↑ROS production	Macrophages (r)	Dimitrijević et al. (2005)
↑NO production	Macrophages (r) and air-pouch granulocytes (r)	Dimitrijevic et al. (2006, 2008)
Y2 receptor		
↑Adherence capacity	T cells	Levite et al. (1998)
	Macrophages (r)	Nave et al. (2004)
↓Phagocytosis and ↑ROS production	Macrophages (r) and granulocytes (h)	Dimitrijević et al. (2005), Bedoui et al. (2008)
↓ROS production	Air-pouch granulocytes (r)	Dimitrijević et al. (2006)
↑NO production	Macrophages (r)	Dimitrijević et al. (2008)
Y5 receptor		
↓Phagocytosis and ↓ROS production	Macrophages (r)	Dimitrijević et al. (2005)
↓ROS production	Air-pouch granulocytes (r)	Dimitrijević et al. (2006, 2010)

h human, *m* mice, *NO* nitric oxide, *r* rat, *ROS* reactive oxygen species

capable of producing NPY encompass monocytes, B lymphocytes and dendritic cells (Lambert et al. 2002; Schwarz et al. 1994).

Endogenous NPY has been related to the autocrine/paracrine regulation of macrophage functions. For instance, the addition of the Y1 receptor antagonist, or Y1 receptor deficiency, reduced the production of cytokines IL-12 and TNF-α in cultured activated macrophages (Wheway et al. 2005). This indicates that NPY produced by the activated macrophages is required for their normal proinflammatory cytokine production. In addition, both Y1 and Y2 receptor antagonists suppressed oxidative burst in rat macrophages, sustaining the involvement of endogenous NPY in the tonic regulation of cellular oxidative metabolism (Nave et al. 2004; Dimitrijević et al. 2005).

NPY and immune-mediated pathological conditions

Infection

It has been shown that increased NPY protein expression in neurons in the vicinity of cells infected with a neurovirulent retrovirus plays a protective role during retroviral pathogenesis in the central nervous system (Du et al. 2010). Conversely, an increased concentration of NPY in cerebrospinal fluid and plasma positively correlated with the degree of HIV encephalopathy, suggesting involvement in the pathogenesis of HIV-related neurological dysfunction (Malessa et al. 1996). The rabies virus did not increase the expression of NPY in neurons, but NPY-expressing neurons were not infected with this virus (Weihe et al. 2008).

Concerning bacterial infection, circulating concentrations of immunoreactive NPY were shown to be markedly raised above the normal range in patients with septic shock (Watson et al. 1988). It has been reported that NPY exerted direct antimicrobial activity against *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Lactobacillus acidophilus* (El Karim et al. 2008b). The antimicrobial properties of NPY are known to be enhanced by N-terminal truncation (Shimizu et al. 1998). NPY was also previously shown to have antifungal activity against *Cryptococcus neoformans*, *Candida albicans*, and *Arthroderma simii* (Vouldoukis et al. 1996). Although the in vivo activity of antimicrobial peptides is influenced by factors such as pH, ion concentration and binding to proteins and glycosaminoglycans, the receptor-specific modulatory effect of NPY on bacterial-induced inflammation is also suggested. Oral diseases such as dental caries and pulp inflammation are driven by the host response to various bacterial species, and Y1 receptors were observed on nerve fibres, blood vessels and inflammatory cells in carious teeth. This suggests a role for NPY via Y1 receptors in the modulation of caries-induced pulpal inflammation (El Karim et al. 2008a). NPY can also modulate IgA secretion following exposure to microorganisms or toxins, as LPS induced NPY production by neurons in the vicinity of IgA-producing lymphocytes in the mouse ileum lamina propria (Shibata et al. 2008).

Inflammation

Increased NPY concentrations were found in the colon and brain of mice with trinitrobenzenesulfonic acid-induced colitis, a model of inflammatory bowel disease (Baticic et al. 2011), but also in dextran sulphate sodium (DSS)-induced colitis, a model of ulcerative colitis (Pang et al. 2010). In spite of the increased NPY levels, segments of proximal and distal colons from mice with DSS-induced colitis were virtually unresponsive to its antisecretory action, expressing less Y1 receptor mRNA and diminished Y1 receptor protein expression in the colonic epithelium (Klompus et al. 2010). Macrophages from Y1 receptor-deficient mice have been shown to produce lower amounts of TNF-α and IL-12 (Wheway et al. 2005). Considering that the DSS model of colitis is highly dependent on the activation of macrophages (Cooper et al. 1993), and that NPY stimulates the proinflammatory activity of macrophages, it is not surprising that Y1 receptor-deficient mice or mice treated with a specific Y1 receptor antagonist displayed reduced clinical and histological signs of acute colitis (Hassani et al. 2005). Y1 receptor deficiency is accompanied by an altered incidence of different immune cell populations in the lymphoid organs, such as an increased number of naïve CD4+ and CD8+ T cells and a decreased number of effector T cells in the lymph nodes (Wheway et al. 2005). Although Y1−/− mice showed impaired Th1 delayed-type hypersensitivity response and insensitivity to DSS-induced colitis, T cells from their lymph nodes were hyperresponsive to anti-CD3/anti-CD28 activation. These findings suggest that an additional mechanism is responsible for the suppression of Th1 responses in Y1−/− mice. Experiments utilising the transfer of Y1−/− lymphocytes into lymphopenic mice revealed a defect in the antigen presenting cell function in Y1−/− mice. This annulled the hyperactive nature of the Y1−/− T cell compartment, and therefore protected Y1−/− mice from Th1 responses. Hindering NPY by antagonists or the NPY antisense oligodeoxynucleotides may be a useful therapeutic approach to treat ulcerative colitis (Pang et al. 2010).

As a result of an augmented release of neuronal NPY, increased plasma NPY levels have been detected in several other inflammatory conditions such as liver cirrhosis (Wiest et al. 2008), asthma (Groneberg et al. 2004), and atopic dermatitis (Salomon and Baran 2008). Considering the substantial direct effects of NPY on the activity of

immune cells, NPY and Y receptors may be a potential target for the modulation of immune-mediated inflammatory diseases.

Autoimmunity

The fact that the development of type 1 diabetes involves the lymphopenia (Lyp) gene, which is closely linked to the neuropeptide Y (Npy) gene on chromosome 4 (Jacob et al. 1992), indicates the direct involvement of NPY in autoimmunity. It has also been recently discovered that NPY might be a possible new minor autoantigen in diabetes mellitus type 1, as one of the secretory vesicle-associated proteins that are targets of the autoimmune response (Hirai et al. 2008).

An abnormal concentration of NPY in different body fluids is a hallmark of several autoimmune-mediated chronic inflammatory diseases. For instance, increased levels of NPY in plasma have been found in systemic lupus erythematosus (Harle et al. 2006), and autoimmune polymyositis and dermatomyositis (Liu et al. 2004). Similarly, rheumatoid arthritis and Sjogren's syndrome are associated with increased NPY levels in synovial fluid and saliva, respectively (Harle et al. 2006; Santavirta et al. 1997). However, the density of NPY-containing nerve fibres is decreased in rheumatoid synovium (Pereira da Silva and Carmo-Fonseca 1990). While the altered levels of NPY expression are not responsible for the initiation of autoimmune diseases, the involvement of NPY in the course of the illness cannot be excluded. In an animal model for human systemic lupus erythematosus, based on the mouse strain that spontaneously develops similar autoimmune aberrations, NPY is significantly increased in the spleen and in the inflamed kidneys in parallel with the progression of the disease (Bracci-Laudiero et al. 1998). Therefore, the elevated local level of NPY in the spleen may be associated with the lymphocyte activation and extensive lymphoproliferation during the development of systemic lupus, thus indicating a role of NPY in maintenance of the inflammation (Bracci-Laudiero et al. 1996b).

In contrast, the concentration of NPY is reduced in the cerebrospinal fluid of patients with multiple sclerosis (Maeda et al. 1994). The possible capacity of NPY to modulate the autoimmune processes associated with multiple sclerosis has been investigated in an accepted experimental rodent model such as experimental autoimmune encephalomyelitis (EAE). In the susceptible rat and mouse strains, EAE is induced either by immunisation with encephalitogen and an adjuvant, or by the passive transfer of CD4+ Th1 lymphocytes obtained from EAE-immunised animals into the naïve recipient. NPY in vivo in a dose-dependent fashion ameliorated the symptoms and severity of the disease in EAE mice (Bedoui et al. 2003b). The beneficial effect of NPY on clinical EAE was linked with the decreased amounts of IFN-γ secreted from autoreactive T lymphocytes when stimulated with the specific autoantigen, and the elevated IgG1-IgG2a ratio of autoantigen-specific antibodies, which is indicative of favouring the Th2 response. Additionally, NPY treatment during the inductive phase of EAE in rats delayed the onset and reduced the severity of clinical signs, and lowered the number of T cells and macrophages infiltrating the brain at the disease peak (unpublished results). Specific Y1 receptor agonists also inhibited the induction of EAE, thus stressing the role of Y1 receptors in the suppressive effect of NPY on EAE (Bedoui et al. 2003b). Furthermore, blocking Y1 receptor signalling by using an antagonist to Y1 resulted in an earlier onset of the disease. The importance of Y1 receptor signalling for disease control is suggested in multiple sclerosis patients. Namely, an increased expression of CD26 molecules (DP4) on T lymphocytes in multiple sclerosis patients (Reinhold et al. 2002) may contribute to a decreased amount of intact NPY, and therefore reduced Y1 receptor signalling. Indeed, the pharmacological inhibition of CD26 activity successfully suppresses the clinical course of EAE, including an active TGF-β1-mediated anti-inflammatory effect on the central nervous system (Steinbrecher et al. 2001).

Majority of studies have underlined the Y1 receptor-mediated suppressive effects of NPY on immune functions. However, some conflicting results require an additional analysis of Y receptor signalling and the mechanisms behind these discrepancies. Studies from Y1 receptor knockout mice have helped to solve some of the controversies concerning the effects of endogenous and exogenous NPY on immune responses. Future investigations should focus more on the involvement of different Y receptor subtypes, and studying immune responses in mice deficient in Y receptors other than Y1 can be of great importance.

Conclusion

Investigations across different cellular compartments revealed that NPY stimulates lymphocyte migration, but generally restrains their ability to proliferate and infiltrate the tissue, and polarises toward Th2 immune activation. All of this is advantageous in Th1-type autoimmune inflammatory disorders. Suppressing the macrophage secretion of inflammatory cytokines and phagocytosis NPY postpones inflammatory reactions. However, by increasing cellular oxidative burst in both macrophages and granulocytes, and by direct bactericidal activity, it facilitates the elimination of pathogens and thus assists in the expansion/termination of already developed inflammatory response. In contrast, NPY reduces NK activity, disputing its overall antimicrobial effects. In addition, endogenous NPY may promote the production of inflammatory cytokines and thus perpetuate

an inflammatory disease, which leads to a debate on whether the association of its increased levels with several immune-mediated diseases may be a result of an overly disrupted immune balance or, alternatively, is more intimately linked to disease initiation. Its wide distribution under physiological conditions and close relationship with the aetiology, pathogenesis or the possible treatment of immune-mediated diseases have revealed NPY as a very intriguing peptide for research in the field of neuroimmunomodulation.

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References

Ahmed AA, Wahbi AH, Nordlin K (2001) Neuropeptides modulate a murine monocyte/macrophage cell line capacity for phagocytosis and killing of Leishmania major parasites. Immunopharmacol Immunotoxicol 23:397–409

Baticic L, Detel D, Kucic N, Buljevic S, Pugel EP, Varljen J (2011) Neuroimmunomodulative properties of dipeptidyl peptidase IV/CD26 in a TNBS-induced model of colitis in mice. J Cell Biochem 112:3322–3333

Beck B, Burlet A, Bazin R, Nicolas JP, Burlet C (1993) Elevated neuropeptide Y in the arcuate nucleus of young obese Zucker rats may contribute to the development of their overeating. J Nutr 123:1168–1172

Bedoui S, Kuhlmann S, Nave H, Drube J, Pabst R, von Hörsten S (2001) Differential effects of neuropeptide Y (NPY) on leukocyte subsets in the blood: mobilization of B-1-like B-lymphocytes and activated monocytes. J Neuroimmunol 117:125–132

Bedoui S, Lechner S, Gebhardt T, Nave H, Beck-Sickinger AG, Straub RH, Pabst R, von Hörsten S (2002) NPY modulates epinephrine-induced leukocytosis via Y-1 and Y-5 receptor activation in vivo: sympathetic co-transmission during leukocyte mobilization. J Neuroimmunol 132:25–33

Bedoui S, Kawamura N, Straub RH, Pabst R, Yamamura T, von Hörsten S (2003a) Relevance of neuropeptide Y for the neuroimmune crosstalk. J Neuroimmunol 134:1–11

Bedoui S, Miyake S, Lin Y, Miyamoto K, Oki S, Kawamura N, Beck-Sickinger A, von Hörsten S, Yamamura T (2003b) Neuropeptide Y (NPY) suppresses experimental autoimmune encephalomyelitis: NPY1 receptor-specific inhibition of autoreactive Th1 responses in vivo. J Immunol 171:3451–3458

Bedoui S, Kromer A, Gebhardt T, Jacobs R, Raber K, Dimitrijević M, Heine J, von Hörsten S (2008) Neuropeptide Y receptor-specifically modulates human neutrophil function. J Neuroimmunol 195:88–95

Berglund MM, Hipskind PA, Gehlert DR (2003) Recent developments in our understanding of the physiological role of PP-fold peptide receptor subtypes. Exp Biol Med (Maywood) 228:217–244

Blundell TL, Pitts JE, Tickle IJ, Wood SP, Wu C-W (1981) X-ray analysis (1.4-Å resolution) of avian pancreatic polypeptide: small globular protein hormone. Biochemistry 78:4175–4179

Bracci-Laudiero L, Aloe L, Stenfors C, Tirassa P, Theodorsson E, Lundberg T (1996a) Nerve growth factor stimulates production of neuropeptide Y in human lymphocytes. Neuroreport 7:485–488

Bracci-Laudiero L, Lundeberg T, Stenfors C, Theodorsson E, Tirassa P, Aloe L (1996b) Modification of lymphoid and brain nerve growth factor levels in systemic lupus erythematosus mice. Neurosci Lett 204:13–16

Bracci-Laudiero L, Aloe L, Stenfors C, Theodorsson E, Lundeberg T (1998) Development of systemic lupus erythematosus in mice is associated with alteration of neuropeptide concentrations in inflamed kidneys and immunoregulatory organs. Neurosci Lett 248:97–100

Bradbury AF, Finnie MD, Smyth DG (1982) Mechanism of C-terminal amide formation by pituitary enzymes. Nature 298:686–688

Burton OT, Zaccone P, Phillips JM, De La Peña H, Fehérvári Z, Azuma M, Gibbs S, Stockinger B, Cooke A (2010) Roles for TGF-β and programmed cell death 1 ligand 1 in regulatory T cell expansion and diabetes suppression by zymosan in nonobese diabetic mice. J Immunol 185:2754–2762

Carlson DE, Le W, Chiu WC, Hoffman GE (2009) Messenger RNA for neuropeptide Y in the arcuate nucleus increases in parallel with plasma adrenocorticotropin during sepsis in the rat. Neurosci Lett 452:146–150

Cavadas C, Silva AP, Mosimann F, Cotrim MD, Ribeiro CA, Brunner HR, Grouzmann E (2001) NPY regulates catecholamine secretion from human adrenal chromaffin cells. J Clin Endocrinol Metab 86:5956–5963

Cooper HS, Murthy SN, Shah RS, Sedergran DJ (1993) Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest 69:238–249

Cooper MA, Fehniger TA, Caligiuri MA (2001) The biology of human natural killer-cell subsets. Trends Immunol 22:633–640

De la Fuente M, Bernaez I, Del Rio M, Hernanz A (1993) Stimulation of murine peritoneal macrophage functions by neuropeptide Y and peptide YY. Involvement of protein kinase C. Immunology 80:259–265

De la Fuente M, Medina S, Del Rio M, Ferrandez MD, Hernanz A (2000) Effect of aging on the modulation of macrophage functions by neuropeptides. Life Sci 67:2125–2135

De la Fuente M, Del Rio M, Medina S (2001a) Changes with aging in the modulation by neuropeptide Y of murine peritoneal macrophage functions. J Neuroimmunol 116:156–167

De la Fuente M, Del Rio M, Victor VM, Medina S (2001b) Neuropeptide Y effects on murine natural killer activity: changes with ageing and cAMP involvement. Regul Pept 101:73–79

Dimitrijević M, Stanojević S, Vujić V, Beck-Sickinger A, von Hörsten S (2005) Neuropeptide Y and its receptor subtypes specifically modulate rat peritoneal macrophage functions in vitro: counter regulation through Y1 and Y2/5 receptors. Regul Pept 124:163–172

Dimitrijević M, Stanojević S, Mićić S, Vujić V, Kovačević-Jovanović V, Mitić K, von Hörsten S, Kosec D (2006) Neuropeptide Y (NPY) modulates oxidative burst and nitric oxide production in carrageenan-elicited granulocytes from rat air pouch. Peptides 27:3208–3215

Dimitrijević M, Stanojević S, Mitić K, Kuštrimović N, Vujić V, Miletić T, Kovačević-Jovanović V (2008) The anti-inflammatory effect of neuropeptide Y (NPY) in rats is dependent on dipeptidyl peptidase 4 (DP4) activity and age. Peptides 29:2179–2187

Dimitrijević M, Stanojević S, Mitić K, Kuštrimović N, Vujić V, Miletić T, Kovačević-Jovanović V (2010) Modulation of granulocyte functions by peptide YY in the rat: age-related differences in Y receptors expression and plasma dipeptidyl peptidase 4 activity. Regul Pept 159:100–109

Du M, Butchi NB, Woods T, Morgan TW, Peterson KE (2010) Neuropeptide Y has a protective role during murine retrovirus induced neurological disease. J Virol 84:11076–11088

Dyzma M, Boudjeltia KZ, Faraut B, Kerkhofs M (2010) Neuropeptide Y and sleep. Sleep Med Rev 14:161–165

El Karim IA, Lamey PJ, Linden GJ, Lundy FT (2008a) Neuropeptide Y Y1 receptor in human dental pulp cells of noncarious and carious teeth. Int Endod J 41:850–855

El Karim IA, Linden GJ, Orr DF, Lundy FT (2008b) Antimicrobial activity of neuropeptides against a range of micro-organisms from skin, oral, respiratory and gastrointestinal tract sites. J Neuroimmunol 200:11–16

Elitsur Y, Luk GD, Colberg M, Gesell MS, Dosescu J, Moshier JA (1994) Neuropeptide Y (NPY) enhances proliferation of human colonic lamina propria lymphocytes. Neuropeptides 26:289–295

Fricker LD (1988) Carboxypeptidase E. Annu Rev Physiol 50:309–321

Friedland JS, Constantin D, Shaw TC, Stylianou E (2001) Regulation of interleukin-8 gene expression after phagocytosis of zymosan by human monocytic cells. J Leukoc Biol 70:447–454

Funkelstein L, Toneff T, Hwang SR, Reinheckel T, Peters C, Hook VYH (2008) Cathepsin L participates in the production of neuropeptide Y in secretory vesicles, demonstrated by protease gene knockout and expression. J Neurochem 106:384–391

Gorrell MD (2005) Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. Clin Sci (Lond) 108:277–292

Groneberg DA, Folkerts G, Peiser C, Chung KF, Fischer A (2004) Neuropeptide Y (NPY). Pulm Pharmacol Ther 17:173–180

Grouzmann E, Comoy E, Bohuon C (1989) Plasma neuropeptide Y concentrations in patients with neuroendocrine tumors. J Clin Endocrinol Metab 68:808–813

Grouzmann E, Fathi M, Gillet M, de Torrente A, Cavadas C, Brunner H, Buclin T (2001) Disappearance rate of catecholamines, total metanephrines, and neuropeptide Y from the plasma of patients after resection of pheochromocytoma. Clin Chem 47:1075–1082

Han S, Yang CL, Chen X, Naes L, Cox BF, Westfall T (1998) Direct evidence for the role of neuropeptide Y in sympathetic nerve stimulation-induced vasoconstriction. Am J Physiol 274: H290–H294

Harle P, Straub RH, Wiest R, Mayer A, Scholmerich J, Atzeni F, Carrabba M, Cutolo M, Sarzi-Puttini P (2006) Increase of sympathetic outflow measured by neuropeptide Y and decrease of the hypothalamic-pituitary-adrenal axis tone in patients with systemic lupus erythematosus and rheumatoid arthritis: another example of uncoupling of response systems. Ann Rheum Dis 65:51–56

Hassani H, Lucas G, Rozell B, Ernfors P (2005) Attenuation of acute experimental colitis by preventing NPY Y1 receptor signaling. Am J Physiol Gastrointest Liver Physiol 288:G550–G556

Hayakawa Y, Smyth MJ (2006) CD27 dissects mature NK cells into two subsets with distinct responsiveness and migratory capacity. J Immunol 176:1517–1524

Hernanz A, Tato E, De la Fuente M, de Miguel E, Arnalich F (1996) Differential effects of gastrin-releasing peptide, neuropeptide Y, somatostatin and vasoactive intestinal peptide on interleukin-1 beta, interleukin-6 and tumor necrosis factor-alpha production by whole blood cells from healthy young and old subjects. J Neuroimmunol 71:25–30

Herzog H, Hort YJ, Ball HJ, Hayes G, Shine J, Selbie LA (1992) Cloned human neuropeptide Y receptor couples to two different second messenger systems. Proc Natl Acad Sci USA 89: 5794–5798

Higuchi H, Yang H-YT, Sabol SL (1988) Rat neuropeptide Y precursor gene expression. mRNA structure, tissue distribution, and regulation by glucocorticoids, cyclic AMP, and phorbol ester. J Biol Chem 263:6288–6295

Hill JW, Elmquist JK, Elias CF (2008) Hypothalamic pathways linking energy balance and reproduction. Am J Physiol Endocrinol Metab 294:E827–E832

Hirai H, Miura J, Hu Y, Larsson H, Larsson K, Lernmark A, Ivarsson SA, Wu T, Kingman A, Tzioufas AG, Notkins AL (2008) Selective screening of secretory vesicle-associated proteins for autoantigens in type 1 diabetes: VAMP2 and NPY are new minor autoantigens. Clin Immunol 127:366–374

Holler J, Zakrzewicz A, Kaufmann A, Wilhelm J, Fuchs-Moll G, Dietrich H, Padberg W, Kuncova J, Kummer W, Grau V (2008) Neuropeptide Y is expressed by rat mononuclear blood leukocytes and strongly down-regulated during inflammation. J Immunol 181:6906–6912

Irwin M, Hauger R, Brown M (1992) Central corticotropin-releasing hormone activates the sympathetic nervous system and reduces immune function: increased responsivity of the aged rat. Endocrinology 131:1047–1053

Jacob HJ, Pettersson A, Wilson D, Mao Y, Lernmark A, Lander ES (1992) Genetic dissection of autoimmune type I diabetes in the BB rat. Nat Genet 2:56–60

Kawamura N, Tamura H, Obana S, Wenner M, Ishikawa T, Nakata A, Yamamoto H (1998) Differential effects of neuropeptides on cytokine production by mouse helper T cell subsets. Neuroimmunomodulation 5:9–15

Kim YW, Kim KH, Ahn DK, Kim HS, Kim JY, Lee DC, Park SY (2007) Time-course changes of hormones and cytokines by lipopolysaccharide and its relation with anorexia. J Physiol Sci 57:159–165

Klompus M, Ho W, Sharkey KA, McKay DM (2010) Antisecretory effects of neuropeptide Y in the mouse colon are region-specific and are lost in DSS-induced colitis. Regul Pept 165:138–145

Kobzik L (1995) Lung macrophage uptake of unopsonized environmental particulates. Role of scavenger-type receptors. J Immunol 155:367–376

Lambert RW, Campton K, Ding W, Ozawa H, Granstein RD (2002) Langerhans cell expression of neuropeptide Y and peptide YY. Neuropeptides 36:246–251

Larhammar D, Wraith A, Berglund MM, Holmberg SK, Lundell I (2001) Origins of the many NPY-family receptors in mammals. Peptides 22:295–307

Levite M (1998) Neuropeptides, by direct interaction with T cells, induce cytokine secretion and break the commitment to a distinct T helper phenotype. Proc Natl Acad Sci USA 95:12544–12549

Levite M (2000) Nerve-driven immunity. The direct effects of neurotransmitters on T-cell function. Ann NY Acad Sci 917:307–321

Levite M, Cahalon L, Hershkoviz R, Steinman L, Lider O (1998) Neuropeptides, via specific receptors, regulate T cell adhesion to fibronectin. J Immunol 160:993–1000

Liu J, Li J, Zhai N, Geng L, Song F (2004) Detection of the levels of neuropeptides, ACTH and cortisol in the blood of patients with polymyositis/dermatomyositis and their significance. J Dermatol 31:392–397

Lundberg JM, Terenius L, Hokfelt T, Goldstein M (1983) High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. Neurosci Lett 42:167–172

Lundberg JM, Saria A, Franco-Cereceda A, Hokfelt T, Terenius L, Goldstein M (1985) Differential effects of reserpine and 6-hydroxydopamine on neuropeptide Y (NPY) and noradrenaline in peripheral neurons. Naunyn Schmiedebergs Arch Pharmacol 328:331–340

Maeda K, Yasuda M, Kaneda H, Maeda S, Yamadori A (1994) Cerebrospinal fluid (CSF) neuropeptide Y- and somatostatin-like immunoreactivities in man. Neuropeptides 27:323–332

Malessa R, Heimbach M, Brockmeyer NH, Hengge U, Rascher W, Michel MC (1996) Increased neuropeptide Y-like immunoreactivity in cerebrospinal fluid and plasma of human immunodeficiency virus-infected patients: relationship to HIV encephalopathy. J Neurol Sci 136:154–158

Medina S, Rio MD, Cuadra BD, Guayerbas N, Fuente MD (1999) Age-related changes in the modulatory action of gastrin-

releasing peptide, neuropeptide Y and sulfated cholecystokinin octapeptide in the proliferation of murine lymphocytes. Neuropeptides 33:173–179

Medina S, Del Rio M, Hernanz A, De la Fuente M (2000a) Age-related changes in the neuropeptide Y effects on murine lymphoproliferation and interleukin-2 production. Peptides 21:1403–1409

Medina S, Del Rio M, Hernanz A, De la Fuente M (2000b) The NPY effects on murine leukocyte adherence and chemotaxis change with age. Adherent cell implication. Regul Pept 95:35–45

Meltzer JC, Grimm PC, Greenberg AH, Nance DM (1997) Enhanced immunohistochemical detection of autonomic nerve fibers, cytokines and inducible nitric oxide synthase by light and fluorescent microscopy in rat spleen. J Histochem Cytochem 45:599–610

Mentlein R (1999) Dipeptidyl-peptidase IV (CD26)—role in the inactivation of regulatory peptides. Regul Pept 85:9–24

Mentlein R, Roos T (1996) Proteases involved in the metabolism of angiotensin II, bradykinin, calcitonin gene-related peptide (CGRP), and neuropeptide Y by vascular smooth muscle cells. Peptides 17:709–720

Michel MC, Beck-Sickinger A, Cox H, Doods HN, Herzog H, Larhammar D, Quirion R, Schwartz T, Westfall T (1998) XVI International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. Pharmacol Rev 50:143–150

Mitić K, Stanojević S, Kuštrimović N, Vujić V, Dimitrijević M (2011) Neuropeptide Y modulates functions of inflammatory cells in the rat: distinct role for Y1, Y2 and Y5 receptors. Peptides 32:1626–1633

Morales-Medina JC, Dumont Y, Quirion R (2010) A possible role of neuropeptide Y in depression and stress. Brain Res 1314: 194–205

Muller S, Weihe E (1991) Interrelation of peptidergic innervation with mast cells and ED1-positive cells in rat thymus. Brain Behav Immun 5:55–72

Mullins DE, Zhang X, Hawes BE (2002) Activation of extracellular signal regulated protein kinase by neuropeptide Y and pancreatic polypeptide in CHO cells expressing the NPY Y(1), Y(2), Y(4) and Y(5) receptor subtypes. Regul Pept 105:65–73

Myers AK, Farhat MY, Vaz CA, Keiser HR, Zukowska-Grojec Z (1988) Release of immunoreactive-neuropeptide by rat platelets. Biochem Biophys Res Commun 155:118–122

Nair MP, Schwartz SA, Wu K, Kronfol Z (1993) Effect of neuropeptide Y on natural killer activity of normal human lymphocytes. Brain Behav Immun 7:70–78

Nave H, Bedoui S, Moenter F, Steffens J, Felies M, Gebhardt T, Straub RH, Pabst R, Dimitrijević M, Stanojević S, von Hörsten S (2004) Reduced tissue immigration of monocytes by neuropeptide Y during endotoxemia is associated with Y2 receptor activation. J Neuroimmunol 155:1–12

Nishizuka Y (1984) The role of protein kinase-C in cell surface signal transduction and tumor promotion. Nature 308:693–697

Nohr D, Weihe E (1991) The neuroimmune link in the bronchus-associated lymphoid tissue (BALT) of cat and rat: peptides and neural markers. Brain Behav Immun 5:84–101

Pandey SC, Zhang H, Roy A, Xu T (2005) Deficits in amygdaloid cAMP-responsive element-binding protein signaling play a role in genetic predisposition to anxiety and alcoholism. J Clin Invest 115:2762–2773

Pang XH, Li TK, Xie Q, He FQ, de Cui J, Chen YQ, Huang XL, Gan HT (2010) Amelioration of dextran sulfate sodium-induced colitis by neuropeptide Y antisense oligodeoxynucleotide. Int J Colorectal Dis 25:1047–1053

Pereira da Silva JA, Carmo-Fonseca M (1990) Peptide containing nerves in human synovium: immunohistochemical evidence for decreased innervation in rheumatoid arthritis. J Rheumatol 17:1592–1599

Petitto JM, Huang Z, McCarthy DB (1994) Molecular cloning of NPY-Y1 receptor cDNA from rat splenic lymphocytes: evidence of low levels of mRNA expression and [125I]NPY binding sites. J Neuroimmunol 54:81–86

Puerto M, Guayerbas N, Alvarez P, De la Fuente M (2005) Modulation of neuropeptide Y and norepinephrine on several leucocyte functions in adult, old and very old mice. J Neuroimmunol 165:33–40

Reich A, Orda A, Wisnicka B, Szepietowski JC (2007) Plasma concentration of selected neuropeptides in patients suffering from psoriasis. Exp Dermatol 16:421–428

Reinhold D, Kahne T, Steinbrecher A, Wrenger S, Neubert K, Ansorge S, Brocke S (2002) The role of dipeptidyl peptidase IV (DP IV) enzymatic activity in T cell activation and autoimmunity. Biol Chem 383:1133–1138

Rethnam S, Raju B, Fristad I, Berggreen E, Heyeraas KJ (2010) Differential expression of neuropeptide Y Y1 receptors during pulpal inflammation. Int Endod J 43:492–498

Romano TA, Felten SY, Felten DL, Olschowka JA (1991) Neuropeptide-Y innervation of the rat spleen: another potential immunomodulatory neuropeptide. Brain Behav Immun 5:116–131

Salomon J, Baran E (2008) The role of selected neuropeptides in pathogenesis of atopic dermatitis. J Eur Acad Dermatol Venereol 22:223–228

Santavirta N, Konttinen YT, Törnwall J, Segerberg M, Santavirta S, Matucci-Cerinic M, Björvell H (1997) Neuropeptides of the autonomic nervous system in Sjogren's syndrome. Ann Rheum Dis 56:737–740

Saurer TB, Ijames SG, Lysle DT (2006) Neuropeptide Y Y1 receptors mediate morphine-induced reductions of natural killer cell activity. J Neuroimmunol 177:18–26

Schwarz H, Villiger PM, von Kempis J, Lotz M (1994) Neuropeptide Y is an inducible gene in the human immune system. J Neuroimmunol 51:53–61

Shibata M, Hisajima T, Nakano M, Goris RC, Funakoshi K (2008) Morphological relationships between peptidergic nerve fibers and immunoglobulin A-producing lymphocytes in the mouse intestine. Brain Behav Immun 22:158–166

Shimizu M, Shigeri Y, Tatsu Y, Yoshikawa S, Yumoto N (1998) Enhancement of antimicrobial activity of neuropeptide Y by N-terminal truncation. Antimicrob Agents Chemother 42: 2745–2746

Simpson KA, Martin NM, Bloom SR (2009) Hypothalamic regulation of food intake and clinical therapeutic applications. Arq Bras Endocrinol Metab 53:120–128

Sipos G, Altdorfer K, Pongor E, Chen LP, Feher E (2006) Neuroimmune link in the mucosa of chronic gastritis with *Helicobacter pylori* infection. Dig Dis Sci 51:1810–1817

Soder O, Hellstrom PM (1987) Neuropeptide regulation of human thymocyte, guinea pig T lymphocyte and rat B lymphocyte mitogenesis. Int Arch Allergy Appl Immunol 84:205–211

Souli A, Chariot J, Voisin T, Presset O, Tsocas A, Balasubramaniam A, Laburthe M, Roze C (1997) Several receptors mediate the antisecretory effect of peptide YY, neuropeptide Y, and pancreatic polypeptide on VIP-induced fluid secretion in the rat jejunum in vivo. Peptides 18:551–557

Stanojević S, Vujić V, Kovačević-Jovanović V, Mitić K, Kosec D, Hörsten S, Dimitrijević M (2006) Age-related effect of peptide YY (PYY) on paw edema in the rat: the function of Y1 receptors on inflammatory cells. Exp Gerontol 41:793–799

Stanojević S, Mitić K, Vujić V, Kovačević-Jovanović V, Dimitrijević M (2007) Exposure to acute physical and psychological stress alters the response of rat macrophages to corticosterone, neuropeptide Y and beta-endorphin. Stress 10:65–73

Steinbrecher A, Reinhold D, Quigley L, Gado A, Tresser N, Izikson L, Born I, Faust J, Neubert K, Martin R, Ansorge S, Brocke S (2001) Targeting dipeptidyl peptidase IV (CD26) suppresses autoimmune encephalomyelitis and up-regulates TGF-beta 1 secretion in vivo. J Immunol 166:2041–2048

Stevens-Felten SY, Bellinger DL (1997) Noradrenergic and peptidergic innervation of lymphoid organs. Chem Immunol 69: 99–131

Straub RH, Mayer M, Kreutz M, Leeb S, Scholmerich J, Falk W (2000) Neurotransmitters of the sympathetic nerve terminal are powerful chemoattractants for monocytes. J Leukoc Biol 67:553–558

Talme T, Liu Z, Sundqvist KG (2008) The neuropeptide calcitonin gene-related peptide (CGRP) stimulates T cell migration into collagen matrices. J Neuroimmunol 196:60–66

Tatemoto K, Carlquist M, Mutt V (1982) Neuropeptide Y—a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 296:659–660

Thompson AC, Justice JB Jr, McDonald JK (1995) Quantitative microdialysis of neuropeptide Y. J Neurosci Methods 60:189–198

Unitt J, Hornigold D (2011) Plant lectins are novel Toll-like receptor agonists. Biochem Pharmacol 81:1324–1328

Vouldoukis I, Shai Y, Nicolas P, Mor A (1996) Broad spectrum antibiotic activity of the skin-PYY. FEBS Lett 380:237–240

Watson JD, Sury MR, Corder R, Carson R, Bouloux PM, Lowry PJ, Besser GM, Hinds CJ (1988) Plasma levels of neuropeptide tyrosine Y (NPY) are increased in human sepsis but are unchanged during canine endotoxin shock despite raised catecholamine concentrations. J Endocrinol 116:421–426

Weihe E, Bette M, Preuss MA, Faber M, Schäfer MK, Rehnelt J, Schnell MJ, Dietzschold B (2008) Role of virus-induced neuropeptides in the brain in the pathogenesis of rabies. Dev Biol (Basel) 131:73–81

Wheway J, Mackay CR, Newton RA, Sainsbury A, Boey D, Herzog H, Mackay F (2005) A fundamental bimodal role for neuropeptide Y1 receptor in the immune system. J Exp Med 202: 1527–1538

Wheway J, Herzog H, Mackay F (2007) The Y1 receptor for NPY: a key modulator of the adaptive immune system. Peptides 28: 453–458

Wiest R, Moleda L, Zietz B, Hellerbrand C, Schölmerich J, Straub R (2008) Uncoupling of sympathetic nervous system and hypothalamic-pituitary–adrenal axis in cirrhosis. J Gastroenterol Hepatol 23:1901–1908

Xapelli S, Agasse F, Ferreira R, Silva AP, Malva JO (2006) Neuropeptide Y as an endogenous antiepileptic, neuroprotective and pro-neurogenic peptide. Recent Pat CNS Drug Discov 1:315–324

Zhou JR, Xu Z, Jiang CL (2008) Neuropeptide Y promotes TGF-beta1 production in RAW264.7 cells by activating PI3K pathway via Y1 receptor. Neurosci Bull 24:155–159

Zukowska-Grojec Z, Vaz AC (1988) Role of neuropeptide Y (NPY) in cardiovascular responses to stress. Synapse 2:293–298

Zukowska-Grojec Z, Karwatowska-Prokopczuk E, Rose W, Rone J, Movafagh S, Ji H, Yeh Y, Chen WT, Kleinman HK, Grouzmann E, Grant DS (1998) Neuropeptide Y: a novel angiogenic factor from the sympathetic nerves and endothelium. Circ Res 83:187–195