Neuropeptide precursors processing in immunocytes : Involvement in Neuroimmune communication.

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Communication and reciprocal regulation between the nervous, endocrine and immune systems are essential for the stability of the organism. Among others, cytokines, hormones and neuropeptides have been identified as signalling molecules mediating the communication between the three systems. Neuropeptides, originally described in the central nervous system (CNS), were also found to be expressed by immune cells and exhibit a number of immunomodulatory properties (Stefano and Salzet 1999; Salzet, Vieau et al. 2000; Blalock 2005) (Elmquist, Scammell et al. 1997). In the last few years various animal models have served to study neuroimmune mechanisms confirming the view of communication between the neuroendocrine and immune systems via neuropeptide signalling (Stefano and Salzet 1999; Salzet, Vieau et al. 2000; Blalock 2005). Another emerging function of neuropeptides within the immune system is their *direct* roles in defence. Peptides with antibacterial properties have been shown to be derived from neuropeptide precursors such as proenkephalin and chromogranin B (Salzet and Stefano 1997; Salzet, Vieau et al. 2000; Salzet 2001; Salzet 2002; Metz-Boutigue, Kieffer et al. 2003). The role of neuropeptide precursors in immunity, through the release of antibacterial peptides, is an entirely novel concept (Day and Salzet 2002). Antibacterial peptides implicated in the innate immune response also derive from processing of "true" proantibacterial peptide precursors like prodefensin (Salzet 2002). Therefore, enzymatic processing, including differential processing events, is a key mechanism to generate an antimicrobial defence in tissues.

The biosynthetic pathway that leads to the production of biologically active neuropeptides begins with the synthesis of large inactive precursor proteins which are cleaved at specific paired or single basic residues within the Golgi secretory pathway (Bergeron, Leduc et al. 2000). It is a family of subtilase-like pro-protein convertases (PCs) that is largely responsible for these processing events that activate precursor proteins into neuropeptides (Bergeron, Leduc et al. 2000). The PCs have been extensively studied in both neural and endocrine systems. However, much less is known concerning their expression, regulation and role within the immune system at the basal level or their function during microbial challenge (Padros, Vindrola et al. 1989; Vindrola, Padros et al. 1990; Vindrola, Mayer et al. 1994; Saravia, Padros et al. 1998). Understanding PC function is important since differential expression of PCs and the resulting cleavage patterns determine the nature and biological activity of the peptide products. Thus, depending on the pattern of PC expression, a single protein precursor can give rise to different peptides with diverse biological activities.

The Pro-protein Convertases (PCs): There are presently seven known genes which give rise to convertases, named furin, PC1/3, PC2, PC4, PACE4, PC5/6 and PC7 (Bergeron, Leduc et al. 2000). There is now a large body of evidence demonstrating that these enzymes are critical for the formation of neuropeptides. In particular, it has been established that PC2 and

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PC1/3 enter the regulated secretory pathway. Thus, these enzymes fulfil a primary prerequisite, since most neuropeptides are assembled and matured within the regulated pathway of secretion. Indeed, in the PC2 null mice (Zhu, Zhou et al. 2002), neuropeptide processing is also severely affected, while the processing of a number of other precursors, such as growth factors and enzymes that transit the constitutive pathway is unaffected. In humans, a naturally occurring PC1/3 gene inactivation results in the impairment of the processing of neuropeptide precursors such as POMC and proinsulin leading to various endocrine disorders (Day and Salzet 2002). These data lead us to the notion that convertases such as PC2 and PC1/3 are tightly linked to the regulated secretory pathway and thus to the biosynthetic pathway of neuropeptides. Quite interestingly, it has recently been shown that two peptides "specifically" expressed in endocrine and neural cells could influence PC2 and PC1/3 activity. The first one, 7B2, is a bifunctional molecule that acts, via distinct domains, both as a chaperone-like molecule and a specific inhibitor of PC2 (Martens, Braks et al. 1994). The second one, proSAAS, has just been characterized and seems to behave as a specific PC1/3 endogenous inhibitor (Fricker, McKinzie et al. 2000; Qian, Devi et al. 2000). The exact function of proSAAS on PC1/3 activity remains to be determined and it cannot be ruled out that, in the same manner as 7B2, it may also act as a chaperone-like molecule.

The Neuroimmune Connection: Neuroimmune interactions can be essentially considered as a bi-directional exchange of information that is carried out by classes of molecules, which were originally thought to be restricted to either neural, endocrine or immune systems (Elmquist, Scammell et al. 1997; Day and Salzet 2002; Blalock 2005). These include neuropeptides such as corticotrophin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), monoamines (epinephrine, norepinephrine and dopamine), glucocorticoids, free radicals, cytokines such as interleukin (IL)-1, IL-6 and Tumor Necrosis Factor (TNF), opioid peptides, opiates and endocannabinoids (Elmquist, Scammell et al. 1997; Stefano and Salzet 1999; Salzet, Breton et al. 2000; Salzet 2001; Day and Salzet 2002; Blalock 2005). Cytokines were originally described as small proteins that coordinate the activity of leukocytes and vascular elements during infection and inflammation. Neuropeptides, originally described in the CNS, were also found to be expressed by immune cells and exhibit a number of immunomodulatory properties (Dantzer 2004; Dantzer 2004). A consequence of these observations has been a comparison of various immunocytes from evolutionarily diverse organisms with neuroendocrine cells (Stefano and Salzet 1999). Immunocytes were shown to bear "receptors" for several neurohormones and hypothalamic releasing factors. In addition, they have the ability to produce neurohormones, after processing by PCs, in response to neuroendocrine and/or immune stimuli (Figure 1, (Stefano, Salzet et al. 1998; Stefano and Salzet 1999)). Such overlapping elements between classic neuroendocrine and immune systems strongly suggest a level of cross talk that is mediated by a common currency of signalling molecules and the key role of processing enzymes such like PCs.

PCs in Immune cells

PC1/3 expression was demonstrated in THP-1 cells (LaMendola, Martin et al. 1997). PC1/3, PC2, and 7B2 are present in macrophages/monocytes, granulocytes, and lymphocytes of the blood and within inflamed sc paw tissue (Mousa, Shakibaei et al. 2004). PC2 and PC1/3 have also been detected in immune effectors organs like spleen (Salzet, Vieau et al. 2000) and their expression increase after bacteria infection (Salzet, Vieau et al. 2000). Immunoelectron microscopy revealed that opioids are localized within secretory granules packed in membranous structures in macrophages, monocytes, granulocytes, and lymphocytes (Mousa, Shakibaei et al. 2004). Endorphin is released by noradrenaline from immune cells *in vitro* indicating that immune cells express the entire machinery required for pro-

opiomelnocortin (POMC) processing into functionally active peptides such as endorphin and are able to release these peptides from secretory granules (Brack, Rittner et al. 2004; Mousa, Shakibaei et al. 2004).

Similarities in expression of enzymes involved in the conversion of neuroendocrine precursors, such as opioids, into functionally mature peptides underscores the potential of human immunocytes to express certain neuroendocrine cell characteristics.

Neuropeptides as source of antimicrobial peptides

Proenkephalin-Derived Antibacterial Peptides:

Secretory granules from adrenal medullary chromaffin cells contain a complex mixture of low molecular mass constituents, such as catecholamines, ascorbate, nucleotides, calcium and several water-soluble peptides and proteins (Metz-Boutigue, Kieffer et al. 2003). These components are released into the circulation in response to splanchnic nerve stimulation. It has long been known that relatively large amounts of proenkephalin and chromogranin-derived peptides are also found in the bovine adrenal medulla. Metz-Boutique's group has shown that antibacterial activity is present within the intragranular chromaffin granule matrix and the extracellular medium following exocytosis (Strub, Garcia-Sablone et al. 1995; Goumon, Strub et al. 1996; Strub, Hubert et al. 1996; Goumon, Lugardon et al. 1998; Metz-Boutigue, Goumon et al. 1998; Goumon, Lugardon et al. 2000; Metz-Boutigue, Lugardon et al. 2000; Lugardon, Chasserot-Golaz et al. 2001; Metz-Boutigue, Goumon et al. 2003; Metz-Boutigue, Kieffer et al. 2003; Briolat, Wu et al. 2005). These peptides inhibit the growth of gram-positive bacteria (*Micrococcus luteus* and *Bacillus megaterium*) at micromolar concentrations. In addition, antibacterial assays on soluble chromaffin granule material, recovered from HPLC, indicate the presence of several other endogenous peptides with potent antibacterial activity against gram-positive and gram-negative bacteria in bovine(Strub, Garcia-Sablone et al. 1995; Goumon, Strub et al. 1996; Strub, Hubert et al. 1996; Goumon, Lugardon et al. 1998; Metz-Boutigue, Goumon et al. 1998; Goumon, Lugardon et al. 2000; Metz-Boutigue, Lugardon et al. 2000; Lugardon, Chasserot-Golaz et al. 2001; Metz-Boutigue, Goumon et al. 2003; Metz-Boutigue, Kieffer et al. 2003; Briolat, Wu et al. 2005). These new antibacterial peptides, derived from the chromogranin B and proenkephalin precursors are stored with catecholamines and released during stress (Metz-Boutigue, Kieffer et al. 2003). Bactericidal activities of the bovine chromogranin B or proenkephalin derived peptides are modulated by the degree of maturation of the precursor and by the presence of post-translational modifications (phosphorylations, O-glycosylation). Natural processing of these precursors at the N- and C- terminals generates most of these peptides (Metz-Boutigue, Kieffer et al. 2003). This points out the key role of adrenals and the processing mechanism present in the chromafin cells. Lack of adrenals dramatically reduces resistance against sepsis, supporting the concept of the involvement of Toll-like receptor in this endocrine tissue. In fact, Toll-like receptors (TLRs) are key elements in the innate immune response, functioning as pattern-recognition receptors for the detection and response to endotoxins and other microbial ligands. Inflammatory cytokines play an important role in the activation of the hypothalamic-pituitary-adrenal HPA axis during inflammation and sepsis. The newly recognized major role of TLR2 and TLR4 and the adrenal stress response during critical illnesses such as inflammation and sepsis demand comprehensive analysis of their interactions(Scocchi, Wang et al. 1997; Scocchi, Wang et al. 1998; Scocchi, Bontempo et al. 1999). Western blot analysis demonstrated the expression of TLR2 and TLR4 in the human adrenocortical cell line NCI-H295(Scocchi, Wang et al. 1997; Scocchi, Wang et al. 1998; Scocchi, Bontempo et al. 1999). Immunohistochemical analysis of normal human adrenal glands revealed TLR2 and TLR4 expression in the adrenal cortex, but not in the adrenal medulla(Scocchi, Wang et al. 1997; Scocchi, Wang et al. 1998; Scocchi, Bontempo et

al. 1999). Considering the crucial role of the HPA axis and the innate immune response during acute sepsis or septic shock, elucidating the functional interaction of these systems should be of great clinical relevance. Moreover, C3H/HeJ mice, lacking Toll-like receptor-4 (TLR-4), and consequently, endotoxin hyporesponsive, have recently been shown to be resistant to glucocorticoid protection against live *Escherichia coli*. Effective antibiotic intervention, as an additional parameter and with concomitant administration of glucocorticoid, not only allows for expected antibiotic protection but also for glucocorticoid protection against *E. coli* or *Staphylococcus aureus* of mice sensitized to tumor necrosis factor alpha, regardless of the status of the TLR-4 receptor. TLRs, including but not limited to TLR-2, may be involved in glucocorticoid protective efficacy against Gram-positive and Gram-negative sepsis(Scocchi, Wang et al. 1997; Scocchi, Wang et al. 1998; Scocchi, Bontempo et al. 1999). Overlapping and possibly endotoxin-independent signaling may become important considerations.

Human leukocytes also contain proenkephalin and chromogranin derived peptide (Tasiemski, Salzet et al. 2000; Tasiemski, Hammad et al. 2002) like peptide B and secretolytin. Levels of both peptides are significantly increased in response to lipopolysaccharides (LPS), surgical trauma and bacterial exposure (Tasiemski, Salzet et al. 2000).

In invertebrates *i.e.* leeches and the mollusk *Mytilus edulis* (Salzet and Stefano 1997; Stefano and Salzet 1999) such a proenkephalin (Tasiemski, Verger-Bocquet et al. 2000)and chromogranin (Tasiemski, Hammad et al. 2002; Salzet and Stefano 2003) derived peptides were detected in hemolymph. Furthermore, invertebrate and vertebrate peptideB/enkelytin was found to be very similar, exhibiting high sequence homology (95-98%)(Tasiemski, Salzet et al. 2000). Peptide B/enkelytin is strongly antibacterial as indicated by *in vivo* and *in vitro* experiments (Tasiemski, Salzet et al. 2000; Salzet 2001; Salzet and Tasiemski 2001). It should be noted that the C-terminus of peptide B contains the opioid heptapeptide Met-enkephalin-Arg-Phe(Tasiemski, Salzet et al. 2000; Salzet 2001; Salzet and Tasiemski 2001). This is similar to what is found in amphibian dermal glands after stress (Amiche, Delfour et al. 1998). There are also a variety of antibacterial peptides and neuropeptides related to opioids (for example dermorphin and dermenkephalin) (Amiche, Delfour et al. 1998). In *C elegans* such a family precursor has also recently been discovered in the genome (Couillault, Pujol et al. 2004). This precursor contains multiple copies of YGGFR or YGGFG peptides as well as antimicrobial peptides containing these sequences (Couillault, 2004 #35). According to genome analyses, drosophila also contains such a precursor (Couillault, Pujol et al. 2004). These peptides seem to be released independently of Toll-like receptor activation (Couillault, Pujol et al. 2004) reflecting a new source of antimicrobial peptides and the ancient origin of such molecules.

A Model of the Unified Neuroimmune Response: Met-enkephalin-Arg-Phe stimulates immunocytes, but does not have antibacterial properties. It binds to the δ_2 opioid receptor subtype (Stefano, Scharrer et al. 1996), as does amphibian dermenkephalin (Amiche, Delfour et al. 1998). Incubation of peptide B with neutral endopeptidase (NEP)-containing immunocytes results in activation of antimicrobial function (Shipp, Stefano et al. 1991), a phenomenon that can be blocked with the NEP inhibitor phosphoramidon (Shipp, Stefano et al. 1991) indicating that it is processed to Met-enkephalin-Arg-Phe. Moreover, an important point for our understanding of the significance of this molecule in diverse organisms is the fact that LPS, surgical trauma and electric shocks directed to neural tissues cause an increase in circulating levels of peptide B/enkelytin and Met-enkephalin. In the past, Stefano's group has demonstrated that electrical shocks stress *Mytilus*, which activates immunocytes via the secretion of Met-enkephalin (Stefano and Salzet 1999). We can now add peptide B to this

stress response. In the aggregate, these data lead us to surmise that the co-processing and liberation of peptide B and Met-enkephalin represents a unified neuroimmune protective response to an immediate threat to the organism, regardless of the specific stimulus (Stefano, Salzet et al. 1998). Since microbial infection often acommpanies physical injury, peptide B is released to deal with real or potential microbial threat, whereas Met-enkephalin stimulates or activates immunocytes during the initial stages of the response. Therefore, this unified neuroimmune response provides a highly beneficial survival strategy at the time it is most needed, at the beginning of the host defense response (Stefano, Salzet et al. 1998). A simultaneous presence of Met-enkephalin is equally important for this response. Both human and invertebrate immunocytes contain δ opioid receptors that appear to mediate activation of these cells (Stefano, Scharrer et al. 1996; Stefano, Salzet et al. 1998; Salzet and Tasiemski 2001). In this regard, Met-enkephalin can be envisioned to activate immunocytes and to provide a chemotactic signal for further immunocyte recruitment (Stefano, Scharrer et al. 1996; Stefano, Salzet et al. 1998; Salzet and Tasiemski 2001). However, this process may take many minutes to accomplish, hence the presence of bactericidal peptide B to act during this period of latency. In this scenario, as peptide B breaks down with time, it first liberates the heptapeptide Met-enkephalin-Arg-Phe or the antibacterial peptide, enkelytin. Met-enkephalin-Arg-Phe can further interact with the δ_2 opioid receptor ensuring a continuation of the immunocyte-activated state, including chemotaxis. This enhancement is required, since human granulocytes and invertebrate immunocytes contain NEP, which appears to be the critical enzyme in this process (Salzet 2001).

Sources of Antibacterial Peptides: In models in which inflammatory processes occur in the absence of bacterial infection, such as during intra-surgical cardiac by-pass, time-course experiments have shown the presence of enkelytin, peptide B, opioids (met-enkephalin and met-enkephalin-Arg-Phe) (Tasiemski, Salzet et al. 2000)and other new antibacterial peptides derived from fribrinopeptide A, chromogranins or aplipoprotein CIII in plasma before surgery (Salzet, unpublished). Their amounts are greatly increased just after surgical trauma (skin incision). Moreover, the peptides identified are also contained within chromaffin granules in cells of the adrenal medulla (Metz-Boutigue, Kieffer et al. 2003). Chromaffin cells are innervated by preganglionic fibers conveyed by the splanchnic nerves, which pass through the celiac and renal sympathetic nerve plexuses and are under the control of stress-sensitive supraspinal centers in the brain (Metz-Boutigue, Kieffer et al. 2003). Thus, the presence and the increase in peptide levels we observed might be due to release of adrenal chromaffin granules induced by a systemic stress response to endothelial injury. Additionally, Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) containing neuronal cell bodies located in the sensory neurons of dorsal root ganglia can be activated by sensory receptors located in the skin. This suggests that incision may also cause the release of these peptides from the adrenal medulla. This is in agreement with the significant increase of these peptides during surgery (Metz-Boutigue, Kieffer et al. 2003). Interestingly, the other source of antibacterial peptides may be the skin itself, possibly from macrophages located in the dermis (Brogden, De Lucca et al. 1996; Brogden, Ackermann et al. 1997; Brogden, Ackermann et al. 1998; Brogden, Ackermann et al. 1999; Brogden, Ackermann et al. 2003; Brogden 2005). In fact, opioids, like several epithelial peptide antibiotics, are constitutively expressed. Others are inducible, either by the presence of microorganisms through yet poorly characterized elicitor receptors, or by endogenous pro-inflammatory cytokines (Nissen and Kragballe 1997; Nissen, Lund et al. 1997). However, considering the fact that monocytes contain pro-enkephalin, we cannot exclude the possibility that autocrine or paracrine pathways also exist to release pro-inflammatory peptides (Nissen and Kragballe 1997; Nissen, Lund et al. 1997). In fact, we recently demonstrated that chromogranin B gene is present in monocytes like proenkephalin

(Figure 2). Its processing into secretolytin is stimulated by IL6 which is released in circulation (Tasiemski, Hammad et al. 2002). Secretolytin is released from monocytes upon IL6 stimulation evocating a systemic action while peptide B (Tasiemski, Hammad et al. 2002) like defensins (Befus, Mowat et al. 1999) would act intracellularly (Figure 3). Neither secretolytin nor peptide B is excreted from neutrophiles (Tasiemski, Hammad et al. 2002).

Other neuropeptides as source of antimicrobial activities.

Another opioid derived peptide, le α-melanostimulating hormone (MSH) is also known to exert an antimicrobial activity (Cutuli, Cristiani et al. 2000; Grieco, Rossi et al. 2003). Alpha-MSH and and its carboxy-terminal tripeptide (11-13, KPV) have been determined to have antimicrobial influences against two major and representative pathogens *i.e. Staphylococcus aureus* and *Candida albicans*. alpha-MSH peptides significantly inhibited *S. aureus* colony formation and reversed the enhancing effect of urokinase on colony formation. Antimicrobial effects occurred over a broad range of concentrations including the physiological (picomolar) range. Small concentrations of alpha-MSH peptides likewise reduced viability and germ tube formation of the yeast *C. albicans*. Antimicrobial influences of alpha-MSH peptides could be mediated by their capacity to increase cellular cAMP. This messenger was significantly augmented in peptide-treated yeast and the potent adenylyl cyclase inhibitor dideoxyadenosine partly reversed the killing activity of alpha-MSH peptides. Reduced killing of pathogens is a detrimental consequence of therapy with anti-inflammatory drugs. alpha-MSH peptides that combine antipyretic, anti-inflammatory, and antimicrobial effects could be useful in treatment of disorders in which infection and inflammation coexist.

Chromogranin precursors contain chromogranin A and chromogranin B, 2 peptides with antibacterial activitie (Strub, Garcia-Sablone et al. 1995; Strub, Hubert et al. 1996; Metz-Boutigue, Goumon et al. 2003; Metz-Boutigue, Kieffer et al. 2003)s. C-terminal processing at basic resides in chromogranin B forms the antibacterial peptide secretolytin and the N-terminal part of chromogranin A contains vasostatin and chromofungin (Strub, Garcia-Sablone et al. 1995; Strub, Hubert et al. 1996; Metz-Boutigue, Goumon et al. 2003; Metz-Boutigue, Kieffer et al. 2003). Chromofungin is an antifungal peptide corresponding to chromogranin A (47-66) which can bind calmodulin in the presence of calcium and induce inhibition of calcineurin, a calmodulin-dependent enzyme (Lugardon, Chasserot-Golaz et al. 2001).Vasostatin-1 and secretolytin, initially present in plasma at low levels, are released just after skin incision (Metz-Boutigue, Kieffer et al. 2003).

Proteolysis and Activation of cationic antimicrobial peptides

Most of the antimicrobial peptides are cationic peptides produced in a propeptide form. Another family of cationic molecules has been demonstrated in vertebrate neutophiles, the cathelicidins (Zanetti, Gennaro et al. 1995). These peptides are a family of antimicrobial peptides are formed like neuropeptides precursors (Zanetti, Gennaro et al. 1995). In fact, initially they are synthesized as prepropeptides with a highly conserved prepro-region of 128-143 residues including a putative 29-30-residue signal peptide and a cathelin-like propeptide of 99-114 residues containing four invariant cysteine residues and a variable C-terminal antimicrobial region ranging in length from 12 to 100 residues(Zanetti, Gennaro et al. 1995). The cathelin domain is similar to cystatin domains present in the precursors of such inflammation-related peptides. Studies of bovine and porcine cathelicidins (Scocchi, Wang et al. 1997; Scocchi, Wang et al. 1998; Scocchi, Bontempo et al. 1999) identified neutrophil elastase as the enzyme that processes cathelicidins in these two animal species. Inhibitors of neutrophil elastase have been used to show that the antimicrobial activity of porcine

inflammatory fluids is dependent to a large extent on the secretion of proprotegrin, a porcine cathelicidin precursor, and its activation by neutrophil elastase.

Another family well known of antimicrobial peptides synthetized in neutrophiles are defensins (Ganz 2004). In fact, during the development of neutrophil precursors in the bone marrow polypeptides with primary antimicrobial function are synthesized, posttranslationally processed and stored in granules. Defensins are 29-50 amino acid antimicrobial peptides that are abundant effector molecules of phagocytes and epithelia involved in host defense (Ganz 2004). Alpha-defensins are a class of defensins expressed in human and other mammalian neutrophils and Paneth cells of the small intestine. They are synthesized as 90-100 amino acid preprodefensins, with a 19 amino acid signal sequence, a ~45 amino acid anionic propiece and a 29-40 amino acid C-terminal mature cationic defensin (Valore and Ganz 1992; Valore, Martin et al. 1996). The removal of the anionic propiece is an activation step that converts the inert prodefensin to antimicrobial mature defensin (Valore and Ganz 1992; Valore, Martin et al. 1996). The enzyme(s) that process prodefensins to defensins in neutrophil precursors have not yet been characterized. The identification of enzymes that convert prodefensins to defensins would greatly advance the study of the biological function of these peptides. The processing of neutrophil defensins bears some resemblance to that of various peptide hormones. Many peptide hormones are processed by SPCs that recognize a dibasic motif N-terminal to the cleavage site, and frequently act together with a carboxypeptidase to liberate the mature active hormone. For neutrophile prodefensins, there is a lysine or arginine in the propiece 0-3 residues from the N-terminus of the mature defensin, suggesting that an enzyme recognizing this cationic site could be involved in the cleavage, perhaps followed by aminopeptidases.

Conclusion

Taken together, these data strongly suggest that the same processing enzymes are expressed in leukocytes as in the neuroendocrine system to accomplish this task. Furthermore, differential processing has also been observed in immune system leading to the production of peptides with biological functions such antimicrobial activities. While there is strong evidence to suggest that the PCs fulfil an important role in the immune system, their functions remain only poorly characterized.

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Figure Legends

Figure 1 : Photomicrographs of *in situ* hybridization study showing expression of (A) SPC3 mRNA in rat spleenocytes within the red pulp and (B) SPC2 mRNA in rat lymph node follicles (see small arrows). With permission of Trends in Neurosciences.

Figure 2 : Photomicrograph of human monocytes in confocal microscopy study showing in red, monocytes expressing chromogranin B-derived peptide and in green, monocytes expressing both chromogranin B-derived (secretolytin) and proenkephalin-derived (enkelytin) peptides.

Figure 3. Precursor processing in vertebrates and invertebrates immunocytes after bacteria challenge.

Immune level : An infection done experimentally provokes the enkephalins synthesis by SPCs attack on neuropeptide precursor to lead neuropeptide derived peptides. Enkephalins induce immunocyte chemotaxis and the release of other signaling molecules (i.e. cytokines), whereas peptideB/enkelytin exert an antibacterial action. Within minutes enkelytin is processed to yield [Met]enkephalin-Arg-Phe that further augments the immunocyte response. Enkephalins also stimulate the Th2 lymphocyte responses via CD3, coupled to Ca2+ intracellular release that conducted to IL4 release. Thus, enkephalins act as immune messengers, so called cytokines. It also stimulates cathelicidin and defensin precursor processing in order to rise antimicrobial peptides in a systemic response. At the present time processing enzymes are unknown.

Figure 1 :

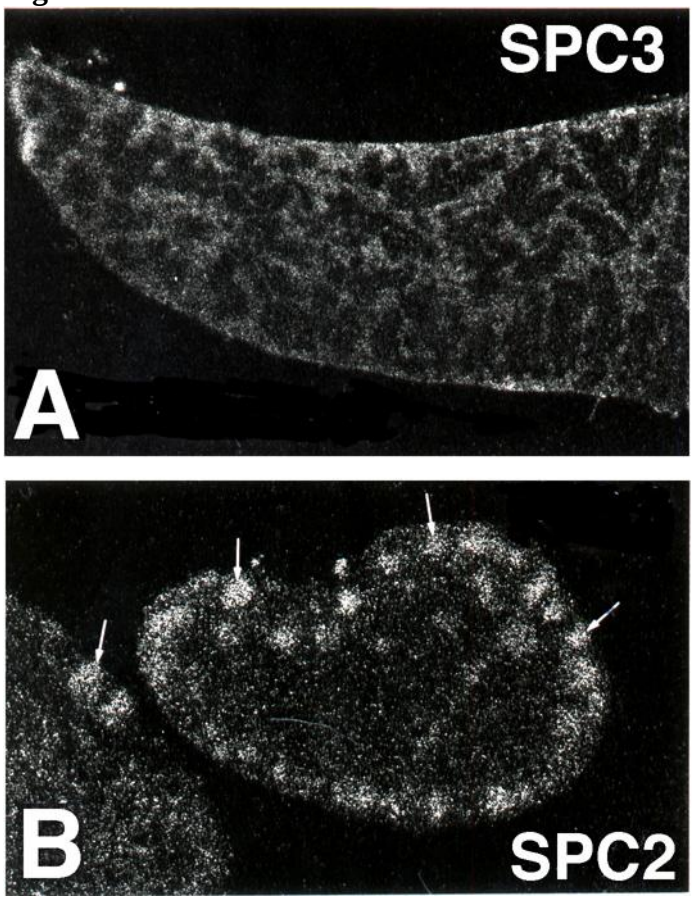

Figure 2 :

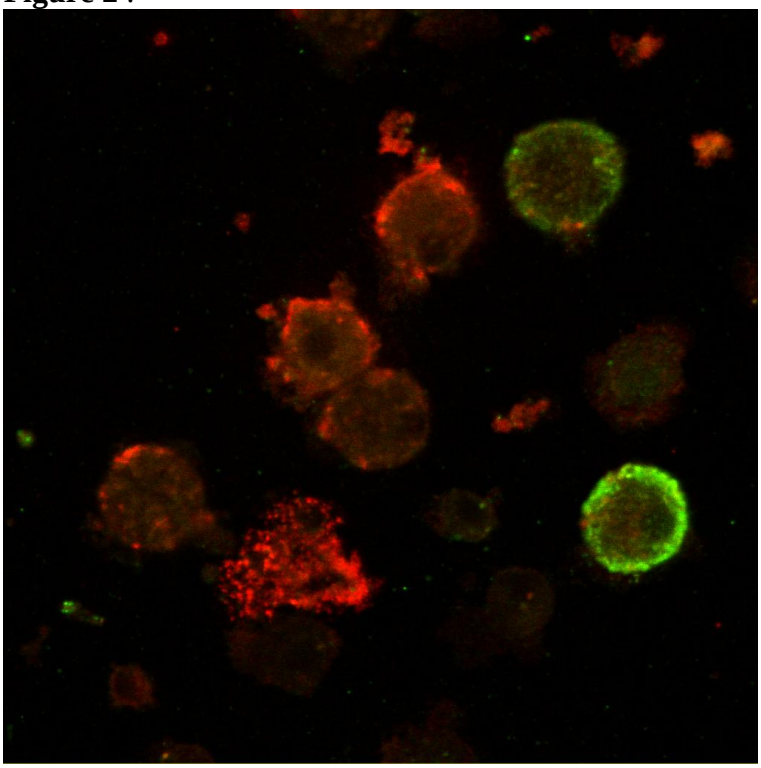

Figure3