The nervous system and innate immunity: The neuropeptide connection

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Kim A. Brogden,[1] Janet M. Guthmiller,[1] Michel Salzet,[2] and Michael Zasloff[3]

[1]Department of Periodontics and Dows Institute for Dental Research, College of Dentistry, The University of Iowa, Iowa City, IA 52252, USA, [2]Laboratoire de Neuroimmunologie des Annelides, UMR 8017 CNRS, IFR 118, Universitedes et Technologies de Lille, 59650 Villeneuve d'Ascq, France, [3]School of Medicine, Georgetown University Medical Center, 3900 Reservoir Road NW, Med-Dent NW 103, Washington, DC 20057. Correspondence should be addressed to K. A. B. (kim-brogden@uiowa.edu).

Summary

Many neuropeptides and peptide hormones are remarkably similar to antimicrobial peptides in their amino acid composition, amphipathic design, cationic charge, and size. Their antimicrobial activity suggests they have a direct role in innate defense. In this review we explore the possibility that the mammalian nervous system, equipped with peptides that exhibit potent antimicrobial properties, utilize neurotransmitters and hormones, in certain settings to directly defend the organism from microbial assault. We focus on several anatomical settings in man in which certain neuropeptides, known to play a critical role in local physiological functioning, could provide a previously unrecognized direct anti-infective, innate immune role in the skin, the gingival crevice of the mouth, the olfactory epithelium, and the adrenal gland.

Considerable evidence has mounted to support active communication between the nervous system and the immune system. The nervous system, including the brain and the peripheral divisions can either stimulate or inhibit various activities of both the innate and adaptive immune systems. Conversely, the immune system, through the release of cytokines, can influence the activity of the nervous system. Several excellent reviews have addressed the subjects of nervous and immune system "cross-talk" in great detail[1-3]. Very recently, however, several peptides, recognized initially for their neural or neuroendocrine signaling functions have been shown to exhibit potent antimicrobial activity. This discovery signals the possibility that the nervous system, through utilization of these peptides, has the capacity to deliver antinfective agents directly to innervated sites, localized with great spatial specificity and delivered rapidly. The nervous and neuroendocrine systems, in principle, have the potential serve a direct immune function.

The skin of most species of frog contains specialized neuro-epithelial structures called granular glands. These structures synthesize and store high concentrations of fully processed, active, antimicrobial peptides (directed at microbes), neuropeptides and neuromuscular toxins (directed at macro-predators). The glands are innervated by adrenergic nerves, which, when stimulated, result in the discharge of the contents of the gland onto the surface of the animal's skin[4]. A dramatic demonstration of this effect can be seen minutes after exposure of an adult African clawed frog (*Xenopus laevis*) to pharmacological doses of noradrenalin, resulting in the massive, simultaneous discharge of all functional granular glands on the skin (**Fig. 1**). In this example, the nervous system is invested with the capacity to directly defend the epithelium of this animal by

discharging a specialized epithelial structure, delivering a potent, highly concentrated cocktail of antimicrobial peptides onto the surface[5].

Clearly, for defensive purposes, frogs use the capacity of the nervous system to respond rapidly to noxious stimuli, and to focus its response at specific sites on the skin surface. In this review we explore the possibility that the mammalian nervous system, equipped with peptides that exhibit potent antimicrobial properties, utilizes these neurotransmitters and hormones, in certain settings, like the example of *Xenopus*, to directly defend the organism from microbial assault.

The blurring definition of antimicrobial peptides, chemokines, and growth factors

Antimicrobial peptides are integral components of innate defense against microbial infection and disease (See article by Selsted, et al in this issue). Over 818 different peptides with antimicrobial activity[6] are produced in many tissues and cell types by a variety of animal, plant, and invertebrate species[7-10].

Antimicrobial peptides were initially discovered as a consequence of their anti-infective activity. However, several antimicrobial peptides have subsequently been shown to exhibit additional biological activities beneficial in the setting of tissue injury and infection (**Fig. 2**). For example, injury and microbial invasion on epithelial surfaces induces certain β-defensins (Selsted, et al in this issue), as well as the local release of neutrophil α-defensins during phagocytosis. The defensins, in turn, can stimulate mast cell degranulation, leading to locally increased vascular permeability, neutrophil accumulation and, induction of epithelial synthesis of the potent neutrophil chemokine,

Il-8. Local inflammatory cascades can be further amplified by the stimulatory effects of neutrophil defensins on the production of macrophage pro-inflammatory cytokines, such as TNF- α, Il- β, and through the inhibition of production of IL-10. This "inflammatory" cascade is further supported physiologically by the anti-glucocorticoid effects of certain neutrophil defensins on glucocorticoid synthesis through occupancy of the ACTH receptor[11]. Communication between defensins and cells of the adaptive immune system can also occur[12]. Neutrophil defensins are chemotactic for resting CD4/CD45RA+ cells and CD8 T lymphocytes. HBD2 (an inducible β-defensin) is a chemoattractant for both memory T cells (CD45R0+) and immature dendritic cells. The effects of HBD2 on these cells is mediated by the human CC chemokine receptor CCR6, the same receptor for which MIP3α is a ligand. LL-37, an abundant broad spectrum antimicrobial peptide of the cathelicidin family, present in both leukocytes and infected epithelial tissues, also exhibits chemoattractant activity for a several cell types including T cells, monocytes, and neutrophils; LL-37 acts specifically on the fMLP receptor, a G protein coupled receptor[13].

Other biological effects of mammalian antimicrobial peptides include stimulation of angiogenesis (**Fig. 2**), induction of epithelial growth factor receptors and co-receptors[14] and stimulation of epithelial division[15-17].

With the discovery that defensins shared receptor binding properties with certain immune cell cytokines, the antimicrobial activity of known cytokines were explored and subsequently discovered. Thus, MIP3α exhibits a more potent antimicrobial activity than HBD2 ; CXCL9,-10, and –11, interferon γ inducible chemokines are active against *E.coli* and *Listeria monocytogenes*[18]; antimicrobial assay of 30 other chemokines, including

members of the CC, CXC, CX3C, and C subfamilies, revealed at least 17 that exhibit antimicrobial activity in vitro[19]. The human platelet stores high concentration of an antimicrobial protein, platelet basic protein (PBP), which is proteolytically processed and secreted upon platelet aggregation; the proteolytic products include truncated forms of NAP2/CXC7, a neutrophil chemokine and CTAP III, both active against *E. coli, S. aureus, C. neoformans*. Recently, at least five other antimicrobial peptides and cytokines, including CXCL4 and CCL5 have been identified in the thrombin activated human platelet[20]. Thus, in addition to its long recognized role in hemostasis, the platelet provides an innate immune function, through its delivery of multifunctional chemokine/antimicrobial peptides to sites of vascular injury; this association in part explains the increased risk of infection associated with thrombocytopenia[20,21].

Making sense of "neuropeptides" with antimicrobial properties

Antimicrobial peptides of multicellular organisms are amphipathic, membrane active molecules that interact with the membranes of a wide spectrum of microbe. Similarly, neuropeptides are generally amphipathic molecules, a property that permits them to achieve high local concentrations within the aqueous space between nerve ending and receptor, and, at the same time, high local concentrations within their target membrane[22]. Indeed, this shared property of amphipathicity served as the motivation behind recent reports of the antimicrobial activities of well studied neuropeptides. Antimicrobial activity, per se, does not alone support the presumed anti-infection function of a peptide. The effective local concentrations achieved must be compatible with the potency, an issue often difficult to evaluate given the paucity of data regarding actual concentrations

of a peptide in situ, and the meaningfulness of antimicrobial activity as measured under the artificial setting of an in vitro assay. Nevertheless, we wish to focus on several anatomical settings in man in which certain neuropeptides, known to play a critical role in local physiological functioning, could provide a previously unrecognized direct anti-infective, innate immune role. The sites we wish to highlight are the skin, the gingival crevice of the mouth, the olfactory epithelium and the adrenal gland.

Substance P

Substance P (SP) is widely distributed throughout the peripheral and central nervous systems. SP is present in a subpopulation of sensory neurons with unmyelinated axons, C-type fibers, which transmit pain ("nocioceptive" stimuli: chemical irritants, injury, heat/cold) from the skin (**Fig. 2**). These fibers have sensory receptors that transmit impulses toward dorsal ganglia, acting as classical afferent nerves; in addition, these same thin fibers can also function as efferent nerves, by releasing release peptides from the same nerve endings that received the noxious stimuli[23]. In the skin SP induces rounding of endothelial cells, relaxation of vascular smooth muscle, leading to capillary leakage and vasodilatation, chemotaxis of neutrophils and macrophages, proliferation of keratinocytes and fibroblasts, mast cell degranulation, and induction of expression of various adhesion proteins on local endothelial, epithelial, and inflammatory cells[24]. SP induces these responses by direct interaction with the NK1 receptor on its target cells, and is deficient in NK1 knock out mice[25], or when effectively blocked by specific antagonists[26]. In addition, the duration of action of an SP stimulus and the extent of its "geographic" impact is constrained by the presence of "neutral endopeptidase", a

protease generally expressed in the local tissue vicinity of SP containing nerve endings[27]. Thus, following a noxious stimulus SP can rapidly set into motion a timed, stereotypically orchestrated defensive scenario even in the absence of actual cellular injury (**Fig. 2**).

SP has been shown in vitro to exhibit antimicrobial activity against *S. aureus, E. coli, E. faecalis, P. vulgaris, Ps. aeruginosa, and C. albicans*[28]. The relative potency is comparable to a potent bactericidal neutrophil antimicrobial peptide indolicidin. The mechanism of antimicrobial activity is not known, but the distribution of amino acids predicts a cationic amphipathic secondary structure comparable to other antimicrobial peptides and likely operating via a common mechanism.

Thus, temporally, the first consequence of the discharge of SP from its nerve endings would be the local infusion of a broad spectrum antimicrobial agent into the tissue space between the nerve endings and the cells bearing NK1 receptors, to be followed by the "slower" unfolding of the NK1-receptor dependent components of the ensuing inflammatory response (**Fig. 2**).

The importance of the neurogenic protection provided by C-type fibers can be appreciated in the setting of diabetes. Sensory neuropathy is an unexplained complication of type I and II diabetes[29]; individuals with sensory neuropathy have a 15 fold greater risk of developing infected foot ulcers requiring lower limb amputation to deal with uncontrollable chronic infection[30]. Although one school of thought argues that these problems arise principally as a consequence of the physical injury due to loss of sensation, recent data suggests that the neuropathy impairs the local neurogenic innate immune defense. Thus db/db mice, like human diabetics, have fewer SP containing

epidermal nerve fibers[31]. Full thickness wounds take a significantly longer period of time to heal in the db/db animals than wild type litter mates, and SP applied to the wounds speeds the healing process in the diabetic animals.

The oral mucosal epithelium has been shown to express defensins, as well as salivary gland histatins, lactoferrin, lysozyme and other proteins with antimicrobial properties[32]. As in other epithelial sites, structures in the oral cavity, including the epithelial surfaces of the oropharyngeal chamber and the tongue, certain microbes and certain cytokines (such as IL-1β) have been shown to stimulate expression of inducible antimicrobial defensins, such as HBD-2. A particularly 'hostile" area of the oral cavity is the region between the tooth enamel surface and the gingival surface - the junctional epithelium (JE) - a physical crevice in which food and microbes can accumulate out of the reach of the abrasive action of the tongue and natural flow of saliva (**Fig. 3**). The JE is a non-keratinized epithelium with a rapid cellular turnover. It is normally infiltrated with neutrophils, even in the absence of inflammation, suggesting these cells accumulate as normal residents of this tissue. The neutrophil based antimicrobial peptides, HNP1 and LL-37 can be localized to the JE and can found in gingival crevicular fluid[33] (**Fig. 3**). Surprisingly, unlike the lingual surfaces of the gingival epithelium and most sites in the oral cavity, the JE itself does not exhibit induction of the inducible antimicrobial peptides, HBD-2 or LL-37, suggesting a dependence on alternative modes of antimicrobial defense. The JE is intensely vascularized with a plexus of venules, and richly innervated with SP containing nerve fibers (**Fig. 3**)[34]. The epithelial cells, vasculature and neutrophils express the NK1 SP receptor. It has been suggested that these SP releasing neurons maintain the "resident" neutrophil population in the JE, via

tonic stimulation[34]. A genetic disorder involving the maturation of myeloid tissues, "Morbus Kostmann" in which the concentration of LL-37 in circulating neutrophils is profoundly depressed, is associated with severe periodontal disease, caused by the commensal microbe. *A. actinomycetemcomitans* suggesting the importance of the neutrophil antimicrobial peptide expression in control of microbial flora in the JE[35].

NPY

Neuropeptide tyrosine (NPY) is a 36 amino acids peptide widely distributed throughout the central and peripheral nervous system[36]. Within the brain NPY is expressed in circuits that affect diverse processes such as feeding, behavior, and energy balance[37,38]. Within the peripheral nervous system, NPY is especially concentrated within the sympathetic division, released from sympathetic nerve endings alone, or along with catecholamines such as epinephrine and norepinephrine. Frequency and duration of stimulation can preferentially release either NPY or CA. Primary and secondary lymphoid tissues are richly innervated by sympathetic nerves containing NPY, and in sites such as the spleen physical evidence of synapses between nerve ending and lymphocytes have been described[1,24]. NPY receptors are present on most of the major cells of the immune system such as macrophages, lymphocytes, and neutrophils[24]. In many settings NPY and α-adrenergic agonists synergize with respect to their effects on immune cells; the effects of CA on the activity of immune cells have been described in detail[1,2]. Amongst the well characterized effects of NPY on immune cells include the inhibition of macrophage release of cytokines such as IL-6, the suppression of the activity

of NK cells, and the inhibition of the generation of specific classes of antibody following exposure to certain antigens[24].

NPY is also synthesized by certain non-neuronal cells of the nervous system[39,40]. In particular, NPY is expressed by a class of glial cells that lie within the olfactory epithelium called olfactory ensheathing cells (OEC) (**Fig. 4**). OEC are a specialized class of glial cells that accompany olfactory sensory neurons as they extend axonal processes from the epithelium through the skull (via the perforated bone called the cribiform plate) ultimately synapsing with nerve endings within the olfactory bulb of the brain. The olfactory epithelium lies at the roof of the nasal chamber of the pharynx. It is exposed to all microbes inhaled through the nose, and provides microbes an anatomical route via the perforated cribiform plate a direct route of entry to the brain. In addition, within the olfactory epithelium are neuronal stem cells, which differentiate continuously throughout life into the olfactory sensory neurons[41]. The olfactory ensheathing cells, which lie immediately beneath the superficial epithelial layer (sustentacular cells) envelop the olfactory neuronal axons, and are believed to both guide the axons and prevent diverting interactions with neural tissue as the axons attempt connection with targets in the olfactory bulb[42]. NPY, secreted by the OECs is believed to act as a growth factor for the olfactory neuron based on in vitro evidence of stimulatory activity and reduced density of olfactory neurons in NPY knockout mice[41].

Precisely what protects the olfactory epithelium from continuous infection, chronic inflammation, and facile microbial entry into the brain remains unexplained although several newly described proteins related to known antimicrobial proteins have been discovered to be expressed in the olfactory epithelium, such as the RY/PLUNC

proteins[43,44], close relatives of the neutrophil bactericidal protein, BPI[45]; these proteins are also secreted by Bowman's glands, a structure which bathes the surface of the olfactory epithelium with a specialized secretion). The recent discovery that NPY has direct antimicrobial activity might also provide a partial explanation for the general "health" of the olfactory tissue. In vitro, NPY was shown to exhibit potent antifungal activity against *C. albicans, Cryptococcus neoformans,* and *Arthroderma simii* and likely activity against Gram negative and Gram positive microbes based on sequence similarity to the homologous potent broad spectrum antimicrobial peptide SPYY, isolated from the skin of the frog, *Phylomedusa bicolor*[46]. The mechanism of action of NPY appears to be similar to other cationic amphiphilic alpha-helical antimicrobial peptides, based on the effects of specific amino acid substitutions on its anti-microbial potency[36]. The expression of NPY by non-neuronal cells within the sustentacular layer, as well as by the olfactory ensheathing cells, might secrete a local "antimicrobial barrier" protecting the projecting axons, discouraging microbial invasion along the axonal tract, and suppressing a need for the assistance of tissue destructive inflammatory cells in the clearance of microbes (**Fig 4**).

Adrenomedullin

AM is a 52 amino acid peptide with a single disulphide bridge, isolated in 1993 from a pheochromocytoma[47]; it is processed proteolytically from a precursor which also contains a second biologically active 20 residue peptide, PAMP, located on the N-terminus of the precursor. Systemic administration of AM or PAMP to mammals, including man, produces vasodilatation resulting in depression of systemic blood pressure through both

direct interaction with specific receptors on the peripheral vasculature as well as with sites within the CNS involved in the regulation of blood pressure[48]. AM is expressed in a wide variety of tissues, but a surprising and unexplained variation in the sets of tissues expressing AM mRNA is observed between species[49]. In humans AM immunoreactivity is found in many diverse tissues including the skin, specific sites within the brain, heart muscle, vascular smooth muscle and endothelium, adrenal medulla, kidney tubular epithelium, and the lumenal mucosal surface and glandular epithelium of the digestive, reproductive and respiratory tracts, as well as in neuroendocrine and endocrine tissues, such as pancreatic islet cells[50]. AM shares a G protein coupled receptor with calcitonin-gene related peptide (CGRP); specificity for either peptide is imparted by the association of a accessory protein, RAMP, of which 3 genes have been identified, RAMPS 1,2, and 3. Association of RAMPS 2 and 3 with the CGRP receptor confers specificity for ADM, while RAMP1 confers CGRP specificity; specific patterns of tissue expression of each of the RAMP genes are observed[51,52].

AM expression in many tissues is strongly induced by the presence of microbes, LPS, or pro-inflammatory cytokines, such as IL-1, as demonstrated both in vitro, and following administration of LPS in vivo[53-55]. With respect to LPS stimulation TLR4 appears to be involved in the stimulation of AM expression in macrophages, since AM expression in cells from C3H/HJ mice following LPS exposure is not observed[53]. It has been suggested that AM might represent a principal mediator of systemic hypotension observed in the setting of bacterial sepsis, prompting the consideration of AM receptor antagonists as potential therapies for this condition[53].

Very recently, both AM and its partner peptide, PAMP, have been shown to exhibit potent microbicidal activity against a wide range of Gram positive and Gram-negative bacteria[56-59]. Commensal and pathogenic organisms that characteristically inhabit specific sites on the human body are killed by concentrations of AM in the 0.5-25 ug/ml range, likely within the range of AM concentrations expressed on the exposed surfaces of the epithelia from these areas. The data strongly suggest that AM has the capacity to provide the sites of expression with broad spectrum, inducible, antimicrobial protection[50,60,61]. The potent activity of both PAMP and AM against organisms such as *P. gingivalis and P. acnes* suggests a role for this molecule in control of growth of these microbes in the gingival crevice of the mouth and the skin, respectively. Indeed, AM can be recovered from the gingival crevice at mg/ml concentrations, well above the minimal bactericidal concentration measured in vitro[62]. In the setting of skin infection and or injury, release of AM from the epidermis would be predicted to stimulate proliferation of keratinocytes, fibroblasts, and produce local vasodilatation (and increased blood flow) through interaction with AM receptors present on these cells and structures[63] (**Fig. 2**). In contrast to both the inducible epithelial defensins and cathelicidins, which are expressed in the more differentiated layers of the epidermis and accumulate within the stratum corneum, AM is present in the most basal layers as well, including the stem cell layer[60,61] (Fig. 1). This would suggest that AM is designed to provide antimicrobial protection to the proliferating population of cells, in addition to the end stage cell protected by other inducible antimicrobial peptides. Its high level of expression by endothelial cells, vascular smooth muscle, and cardiac muscle, also suggests AM might well play a direct role in antimicrobial defense of the blood vessel walls and the heart.

αMSH

α-Melanocyte stimulating hormone (αMSH), is a 13-amino acid peptide produced through post-translational processing of pro-opiomelanocortin (POMC); POMC is cleaved into at least 5 peptides, including the MSH group (α, β, γ), adrenocoticotrophic hormone (ACTH), and β-endorphin. These peptides are secreted by pituitary cells, astrocytes, monocytes, melanocytes, and keratinocytes and are found in the skin and intestinal tract of rats and humans[64]. Five subtypes of melanocortin receptor have been identified to date distributed in the brain and in peripheral tissues; they are G-protein coupled receptors involved in the transmission of physiological responses that include stimulation of melanocyte pigmentation (MC1 receptors), inhibition of food intake (MC4 receptors), and potent suppression of inflammatory processes (possibly MC1, MC3, and MC5 receptors)[65]. Indeed, systemic administration of αMSH or synthetic analogues exhibit activity in animal models of local and systemic inflammation, including rheumatoid arthritis, inflammatory bowel disease, encephalitis, and experimental autoimmune uveitis[66-68]. These effects are mediated, in part, through interaction of αMSH with MC receptors on cells involved in the inflammatory response. αMSH suppresses TNF-α production by activated monocytes and reduces expression of CD86, a costimulatory molecule; αMSH inhibits neutrophil chemotaxis and inhibits the production of pro-inflammatory cytokines from many types of human cell following stimulation by lipopolysaccharide ; αMSH inhibits the LPS-induced activation of VCAM-1 and E selectin in human microvascular cells[68]. A universal mechanism

involving MSH inhibition of NFkB stimulation has been postulated to underlie its broad anti-inflammatory property[69,70].

Current views of the properties of αMSH in human skin are informative. Levels of receptor are low in normal keratinocytes. UV radiation, or pro-inflammatory cytokines such as IL-1, stimulate the keratinocyte's and melanocyte's production of both αMSH and the MCR1 receptor[71-73]. Hence, in human skin, αMSH appears to exert its anti-inflammatory effects following pro-inflammatory or injurious stimuli. αMSH stimulates human epidermal melanocytes to take on a dendritic shape, required for transfer of pigment to keratinocytes. αMSH stimulates eumelanin (dark brown) synthesis more robustly than it does the yellow-reddish pigment, pheomelanin; eumelanin is more photoprotective for cellular DNA against UV radiation than pheomelanin, and accumulates in "eumelanosomes" which organize in the melanocytes and keratinocytes in "sun-protective"supranuclear caps (**Fig. 2**).

Recent studies have demonstrated that αMSH has potent anti-microbial activity[64,74]. Although activity against *S. aureus* was demonstrated, the activity against *Candida albicans* was more extensively studied with respect to mechanism. In the case of *C. albicans*, αMSH appears to have potency in vitro comparable to fluconazole. Both the net cationic charge and hydrophobicity of the peptide appear to be important, a property shared with other antimicrobial peptides, reflecting, presumably this peptide's binding to a membrane target. However, in the case of its candidacidal activity αMSH appears to act through a mechanism that involves increasing intracellular concentrations of cAMP, a response that also occurs in mammalian cells upon binding of αMSH to its receptor. In addition αMSH and selected analogues inhibit canididal germ-tube formation induced by

serum, and stimulate neutrophil-mediated killing of yeast forms. Significant in vitro inhibitory effects can be observed at concentrations (10^{-15} to 10^{-12} M) far below those observed for the majority of known antimicrobial peptides. Surprisingly, the C-terminal tripeptide sequence KPV independently exhibits much of the activity of the complete 13 amino acid peptide, in vitro. Octapeptides comprising variations on the MSH sequence (6-13) have been synthesized with enhanced anti-fungal activity[74].

The suppressive effects on cytokine synthesis and other cellular properties of certain circulating human cells suggested αMSH might have an inhibitory effect on the replication on viruses that propagate in cells that bear MC receptors. Indeed, αMSH was shown to inhibit the replication of HIV in acutely infected human monocytes and in a line of chronically infected human promonocytes[75].

Proenkephalin A

PEA is a precursor of the enkephalin opiod peptides, proteolytically processed to yield several biologically active peptides including Met-enkephalin, Leu-enkephalin, Met-enkephalin-Arg-Phe, Met-enkephalin-Arg-Gly-Leu; enkelytin; and proenkephalin A-derived peptides (PEAP) like Peptide B[76]. In addition to specific sites within the brain, PEA is expressed in certain cells of the immune system and the adrenal medulla. Following administration of LPS to a rat, levels of PEA mRNA rise rapidly in monocytes and macrophages within lymph nodes and within the chromafin cells of the adrenal medulla[77]. Within several hours exceeding the tissue concentration of this mRNA in the hypothalamus (where induction is not observed) by at least 10 fold. Other opioid peptide genes, such as POMC and dynorphin, were not induced. The physiological significance

of the responsiveness of the PEA gene in these sites remains unclear, but effects of enkephalins on various activities of immune cells (chemotaxis, cytoxicity, immunoglobulin synthesis) are supported by the presence of opiod receptors on various immune cells[78]. The possibility that PEA derived peptides provide local or central analgesia as part of the host response to infection has also been proposed[79].

The concentration of PEA protein within the unstimulated adrenal medulla (bovine) exceeds 200μg/gm of chromaffin granule protein [80]. Stimulation of the autonomic nerves innervating the adrenal gland leads to secretion of catecholamines, peptides like the encephalins, NPY, VIP, and, proteolytic fragments of peptide precursors as well as fragments of proteins called chromogranins. Recently, peptides with antimicrobial activity but no known neuropeptide function have been identified in the adrenal medullary chromaffin cell discharge. They include enkelytin and Peptide B from PEA[81], and fragments from Chromogranin A (vasostatin[82], chromofungin[83]) and Chromogranin B (secretolytin[84]), an antifungal peptide. These peptides can be found in the human, minutes following surgical procedures[85]; physiological stimuli associated with surgery presumably route through the sympathetic nervous system to the adrenal medulla and lead to release of chromaffin cellular contents. Antimicrobial PEA fragments have also been identified in abscess fluids, presumably, in this case, of immune cell origin[81].

The precise role played by the antimicrobial peptides that can be generated within the adrenal medulla remains uncertain, but PEA and chromogranin concentrations are sufficiently high for us to entertain the possibility that antimicrobial peptides generated from these proteins might serve to provide local antimicrobial protection within the

adrenal medulla itself, following activation of the sympathetic nervous system, or release of LPS into the intravascular space. The loss of the adrenal gland secondary to infection, is a lethal medical crisis associated with shock. However, except in the setting of meningococcemia, infection or inflammation of the adrenal gland is a medical rarity. The non-inflammatory mechanisms that defend the adrenal gland from blood borne microbes remain unknown.

Concluding Remarks

The inclusion of neuropeptides into the armentarium of antimicrobial peptides extends the known mechanisms by which the nervous system can influence immune function. The extent to which antimicrobial neuropeptides are utilized as we have proposed in this review, such as in the oral cavity or the olfactory epithelium, awaits definitive experimental support. Were it possible to control the anti-infective functions of innate immunity by manipulations of the nervous system, as "simply" as we can now adrenergically stimulate the African clawed frog to release a protective shield of antimicrobial peptides over its skin, new therapeutic avenues for the treatment and prevention of infectious disease in man would open.

Figure Legends

Figure 1. Discharge of antimicrobial peptide rich secretions. Powdered noradrenaline (about 10 mg) was rubbed gently over the dorsal surface of an adult female *Xenopus laevis*. Within 10 minutes most of the dorsal granular glands have discharged their contents.

Figure 2. Antimicrobial peptides expressed in human skin. The cartoon illustrates only a limited view of the known interactions between various peptides and the cells and tissues in the skin. Each panel illustrates a published immunohistochemical study: LL-37, psoriasis skin. (Figure 3, Frohm M et al, J Biol. Chem. 272:15258-15263(1997); HBD2, psoriasis (Ali et al, J of Invest Derm 117:106(2001); Adrenomedullin, normal (Figure 1, Martinez A et al, ibid); α MSH, normal(Figure 1, Nagahama M et al, Brit Journal of Dermatology 138:981-995(1998); Substance P, normal (Figure 1, Pergolizzi S, et al. Archives Derm Res 290:483-489(1998). Cutaneous nerves have been immunolocalized with antibody to PGP 9.5 and are stained white. N, neutrophil; MC, mast cell; MP, macrophage; iDC, immature dendritic cell; TC, T lymphocyte.

Figure 3. Substance P and antimicrobial peptides of the gingival sulcus of the oral cavity. Upper left panel: Defensin expression in human gingival tissue.(Figure 5, from Dale et al, 2001, ibid.) HBD1(constitutive) and HBD2 (inducible) are epithelial defensins; HNP1 is of neutrophil origin. Thin arrows mark borders of gingival tissue; the lower half of the inset in panel b contains the junctional epithelium (JE). HBD1 and

HBD2 expression is weak in the JE. Neutrophils provide the major source of defensins (and LL-37) to the JE). Lower left panel: Substance P containing nerves in the rat gingival sulcus (from Figures 1,2 Kido MA et al, ibid) Fig 1, low power; Fig 2, high power (bar = 10 microns); OE, oral epithelium; ES, enamel surface; OSE, oral sulcular epithelium. JE is densely populated by SP containing nerves.

Figure 4. NPY producing cells in the murine adult olfactory system. Expression of NPY in non-neuronal sustentacular cells of the olfactory epithelium and olfactory ensheathing cells below the epithelial layer (from, Figure 1, Hansel DE et al, Ibid.) NST, neuron-specific tubulin, a marker of olfactory sensory neurons; SC, sustentacular cells. The experiment demonstrates that NPY is synthesized by a non-neuronal cellular population. Cartoon has been modified from Ubink et al, 2003 ibid.

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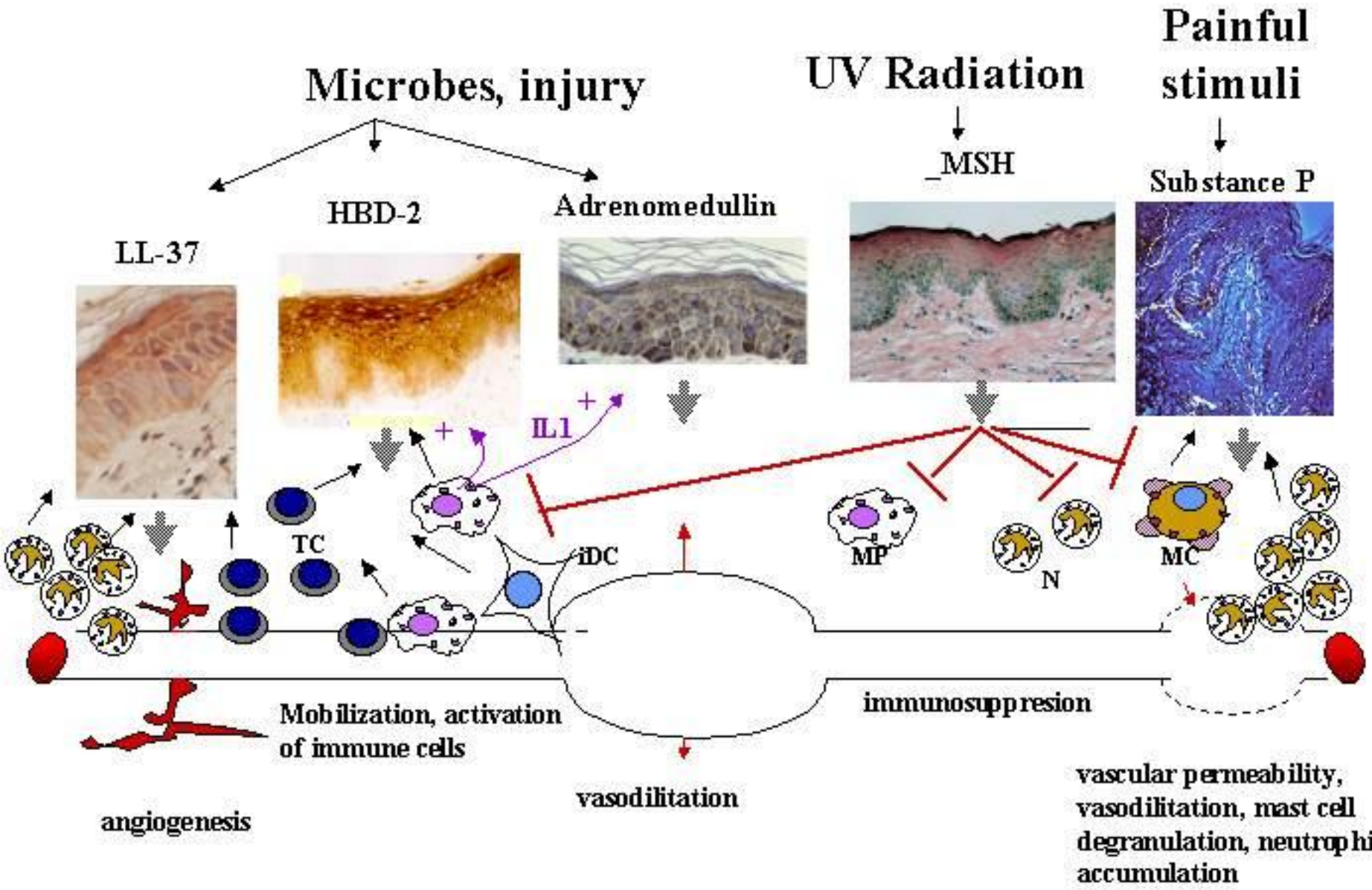
Microbes, injury
UV Radiation
Painful stimuli
_MSH
Substance P
HBD-2
Adrenomedullin
LL-37
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Mobilization, activation of immune cells
immunosuppresion
vasodilitation
angiogenesis
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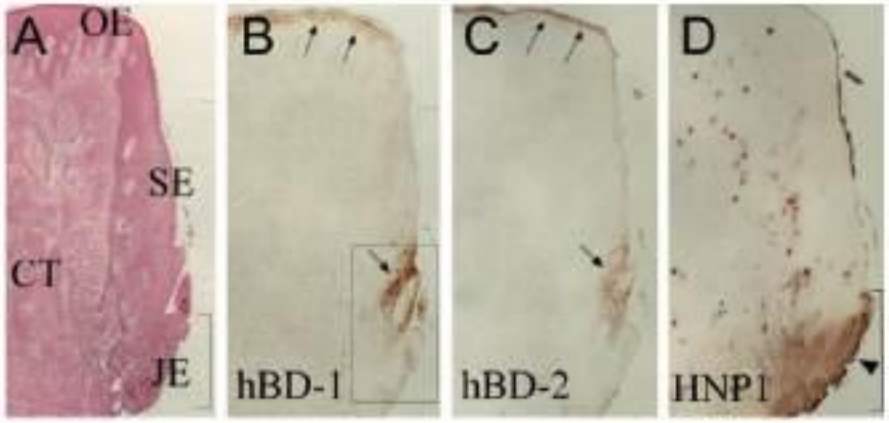
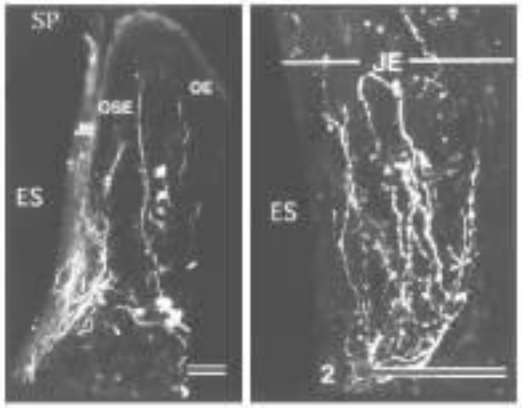
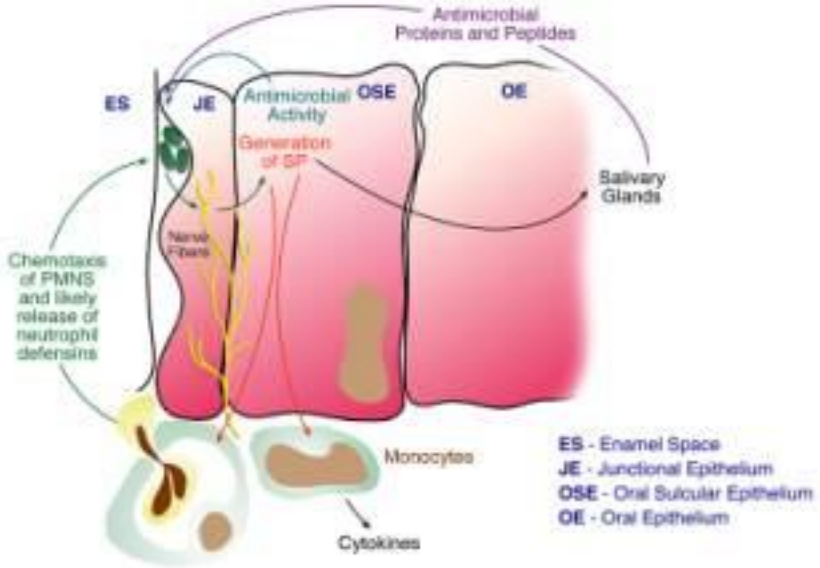


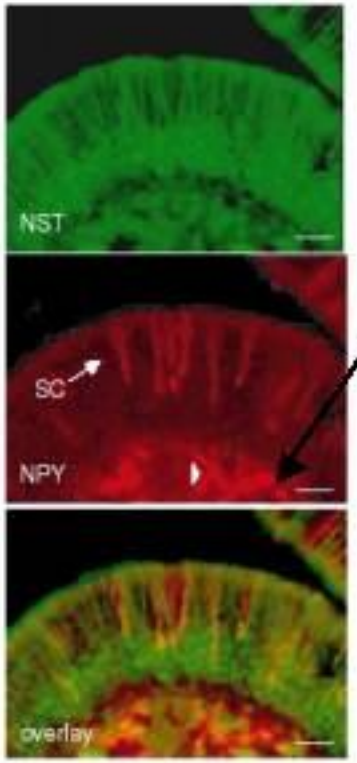
Defensin expression in the gingival sulcus



SP fibers in the junctional epithelium



NPY is expressed in non-neuronal sustenacular cells and sub-laminar glia of the olfactory epithelium



Olfactory epithelium

NPY producing olfactory ensheathing cell

nose

Sensory neuron

olfactory bulb

Cribiform plate of the skull

NPY-producing Sustenacular cell

NPY

microbes