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Antimicrobial peptides in the brain: neuropeptides and amyloid

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1. ABSTRACT

Antimicrobial peptides (AMPs) are ancient defense molecules of the innate immune system. Similarly, neuropeptides are ancient signaling molecules. Similarities in size, cationic charge or amphipatic design between some neuropeptides and AMPs suggest that they might serve an additional function in antimicrobial immunity. This hypothesis, supported by experimental evidence, adds another level of understanding to the intricate crosstalk between the nervous system and the immune system. The recent observation, that another brain protein, amyloid-beta, has antimicrobial activities, suggests that this peptide, prominently known as an accumulating toxic waste material, might have a physiologic function as anti-infective agent.

2. INTRODUCTION

Simple organisms rely on innate immune defense mechanisms, like detection of pathogen associated molecular patterns (PAMPs) by Toll-like receptors (TLRs) or lectins. Simpler defense mechanisms also involve antimicrobial peptides (AMPs), which are either induced at the time of infection or released from stores in secretory cells (1). AMPs are widely distributed in nature and can be considered as the most basic defense mechanism of the innate immune system. Even in humans, there are many AMPs which protect the body against external invading microorganisms. Notably the brain, which is shielded by the blood-brain barrier (BBB) against products of the adaptive immune system, like antibodies, largely relies on local innate immune defense. AMPs can be found in a

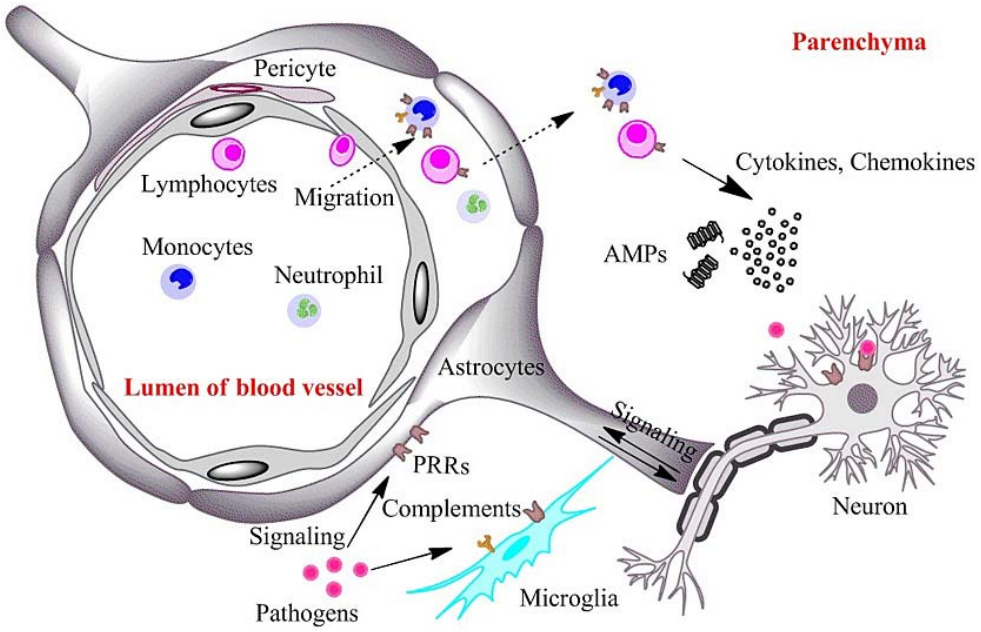

Figure 1. Schematic representation of pathological processes during neuroinflammation. In the normal brain, the blood-brain barrier is shielding the brain against leukocytes. Attack by pathogens results into the recognition of PAMPs by microglial and other cell types. A local signaling cascade affects specific areas of the blood vessel, the postcapillary venules. At these inflammatory sites, lymphocytes and macrophages can transmigrate into the brain parenchyma, secreting more immune mediators, amplifying inflammation, eradicating infection and – in the best case scenario – resolving the lesion by regulating regeneration and proper tissue remodeling.

variety of different cell types in brain. These AMPs include defensins, cathelicidins, and dermcidin (DCD). However, they are not unique to the brain (2, 3).

CNS-resident cells such as microglia, astrocytes and even neurons, are major contributors to the cellular innate immune system of the brain. They sense pathogens and launch the innate immune response (4) Figure 1. Microglial cells communicate reciprocally with astrocytes, endothelial cells and CNS-infiltrating immune cells (5). It has also been suggested that even neurons participate in immune defense of the CNS by signaling to microglial cells (6). Furthermore, leukocytes, such as neutrophils, macrophages, T- and B- and natural killer cells, are major contributors to inflammation of brain. Peripheral immune cells can migrate into the brain by transmigration at postcapillary endothelial venules across the BBB following the inflammatory signals. These infiltrating inflammatory cells affect the course of CNS diseases and potentially serve as the major contributors to neuropathological processes (7, 8).

In this scheme, an intricate signaling network, including almost all cell types of the CNS, from barrier forming endothelial cells, pathogen sensing pericytes, astrocytes and their foot-processes, microglial cells and oligodendrocytes, and neurons, connects CNS cells to the inflammatory reaction. Thus, inflammation is integrated into parenchymal brain cell signaling and since the immune system and the nervous system coevolved, it could be anticipated that inflammatory signals affect neural cell function and neural signals immune cell function.

There is a large volume of experimental data on this interesting topic. However, in this review we have selectively considered AMPs and their relation to neuropeptides. AMPs are evolutionary ancient weapons and are known as conserved essential components of congenital, innate immunity, which is the principal natural defense system for the majority of living organisms, and are found among almost all classes of life ranging from prokaryotes to humans. Not surprisingly, AMPs have been considered clinically useful antibiotics (3, 8-10), overcoming the problem of emergence of multidrug-resistant pathogens. However, current progress is not meeting expectations in this respect.

AMPs were first isolated in the 1980s (1, 11). Up to now, a wide variety of AMPs has been isolated from nature and thousands of synthetic variants have been produced. Hundreds of AMPs from plants and animals have been described and the number is still adding up (2-4). For a regularly updated list of plant and animal AMPs, see the website: http://www.bbcm.univ.trieste.it/~tossi/antimic.html. In mammals, many AMPs can be found in immune cells as well as the cells associated with body surface defense. Many studies have also detected AMPs in the brain tissue (4, 12-15).

3. NEURO-ANTIMICROBIAL PEPTIDES (NAMPs)

The functions of neuropeptides range from neurotransmitter to growth factor. They are present in glial

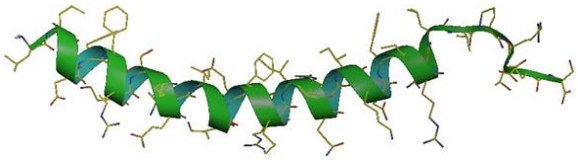

Figure 2. Protein structure of human cathelicidin LL-37.

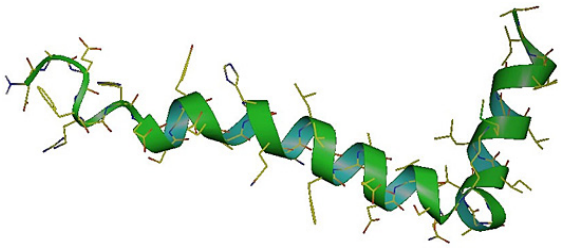

Figure 3. Protein structure of Alzheimer's disease amyloid beta-peptide.

cells; act as hormones in the endocrine system, or as messengers in the immune system. Neuropeptides are of particular importance when the nervous system is challenged (e.g., by stress, injury, or drug abuse), and they are involved into different stages of a proinflammatory response as signaling molecules. Many neuropeptides share similar characteristics with AMPs in their amino acid composition, amphipathic design, cationic charge, and size, which facilitate their deposition into the aqueous space between the target membrane and within the nerve terminal and respective receptor. This shared property of amphipathicity led to discovery of the antimicrobial activities of many neuropeptides (12-16).

The term "NAMP" (neuro-antimicrobial peptide) can be coined as an AMP derived from neuropeptides. The inclusion of neuropeptides into the armentarium of antimicrobial peptides extends our knowledge of mechanisms by which the nervous system contributes to innate immune defense. Several peptides with neural or neuroendocrine signaling functions have been shown to have potent antimicrobial activity. Furthermore, they are widely distributed not only in endocrine, neuroendocrine and nerve cells, but also in immune cells. The antibacterial effects of NAMPs can be bacteriostatic or bacteriocidal, depending on concentration and types of pathogenic bacteria.

For example, Proenkephalin A (PEA) is expressed at specific sites in the brain and certain cells of the adrenal medulla and the immune system. It is the precursor of the enkephalin opioid peptides and is proteolytically processed to yield Met-enkephalin, Leu-enkephalin, enkelytin and PEA-derived peptides such as peptide B (14-17). Enkelytin is a major PEA-derived peptide and is active on gram-positive bacteria including *Staphylococcus aureus* at the micromolar range.

Neurokinin-1 (NK1; also known as substance P) is widely distributed throughout the peripheral and central nervous system. Like other AMPs, NK1 has a cationic amphipathic secondary structure and probably shares a common mechanism of antimicrobial activity (18).

Bombesins comprise a large family of neuropeptides mediating a variety of stimulation of biological activities in the gastrointestinal (GI) tract and central nervous system (CNS) of mammals. These activities include contraction of smooth muscle, secretion of GI hormones, processing of memory, enhancement of cell proliferation, and regulation of central homeostatic mechanisms (appetite and feeding behavior, thermoregulation). Proline-rich bombesin, originates from Chinese red belly toad of *Bufo maxima*. It is a 16-amino acid peptide member of bombesin-related peptides (BRPs) family. Like substance P (SP) and neuropeptide Y (NPY), PR-bombesin might also have direct effects on invading microbes. These antimicrobial activities add a further dimension to the immunomodulatory roles of neuropeptides in inflammatory and immune responses. This discovery suggests that the nervous system might use these peptides as anti-infectious agents by delivering them rapidly and precisely to innervated sites (19).

4. AMYLOID-BETA AS AN AMP, LL-37

Recently, it has been shown that another neuropeptide, amyloid-beta, has antimicrobial activities and has to be considered an AMP, although it has, unlike many other AMPs, an anionic and not a cationic overall charge (20-23).

Beta-amyloid, a processing product of amyloid precursor protein (APP) is considered an unwanted toxic protein, accumulating in the Alzheimer's brain plaques as a largely insoluble waste. Surprisingly, amyloid-beta has many different biological activities, like binding to transport pumps and being a ligand to various receptors. Notably, amyloid-deposition is associated with inflammation and microglial cell accumulation. Thus, amyloid-beta could be considered a product of an aberrant cellular response to an invading infectious agent. Amyloid fibers can also be formed by a variety of other immune proteins, like lysozyme or protegrin-1, and the innate immunity receptor TLR2 is able to recognize amyloid fibers (22, 24, 25). It is possible that the precipitation of amyloid is an attempt of local immunity to encage and permanently trap infectious organisms.

The antimicrobial activity of amyloid-beta has been traced to similarities in structure with the human AMP LL-37 (21, 26, 27), which can be produced by astrocytes. In the following, we present some observations concerning these similarities.

The protein structures of human cathelicidin LL-37, based on protein data bank 2K6O, and Alzheimer's disease amyloid beta peptide, based on protein data bank 1IYT, are respectively shown in Figure 2 and Figure 3. In both cases, the solution NMR has been employed in order to measure the protein structure. The human cathelicidin LL-37 consists of 37 amino acids in its primary structure which is formed into an alpha-helix with a flexible N-terminal (28). In contrary, the presented structure of Alzheimer's disease amyloid beta

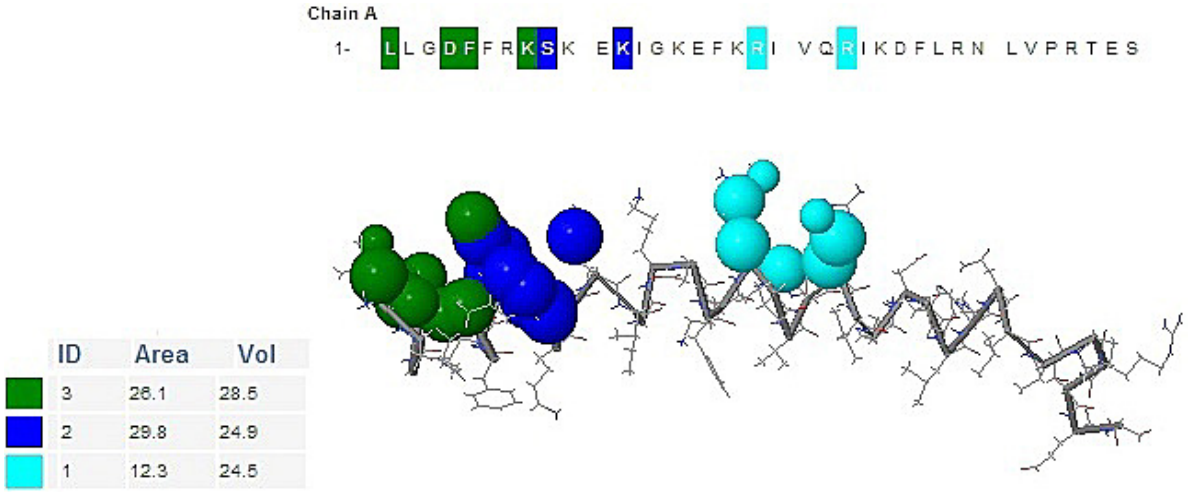

Figure 4. Protein pockets of human cathelicidin LL-37 determined by castP analysis. Different colors present different pockets. The amino acids involved in each pocket are also shown with the same color in the protein sequence. The embed table gives the values of area and volume of each pocket in angstrom scale.

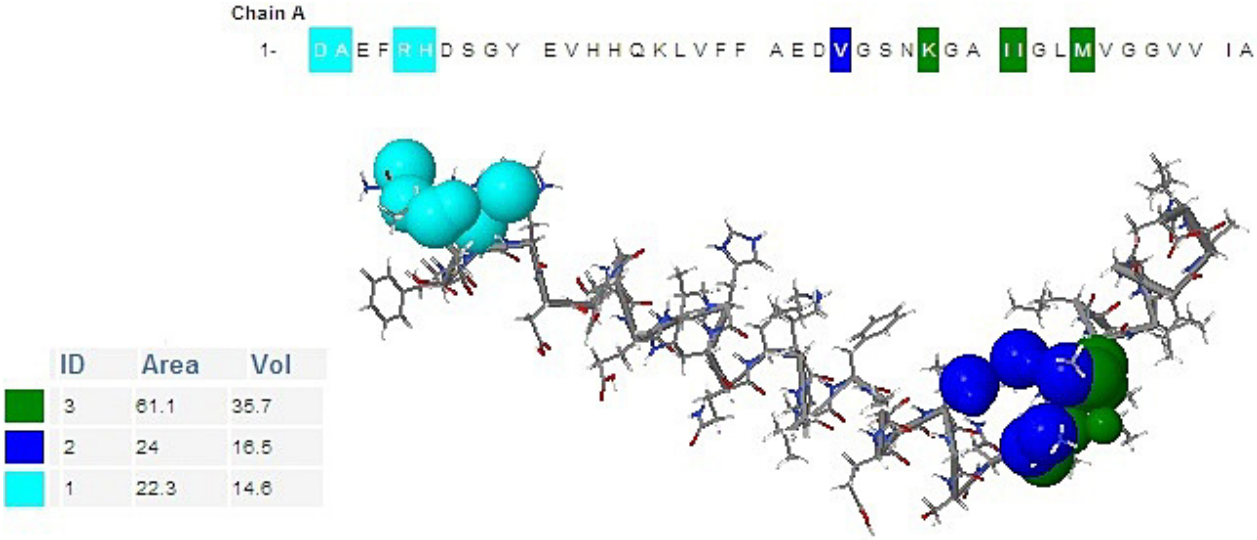

Figure 5. The same as Figure 4, but for Alzheimer's disease amyloid beta-peptide.

peptide, consisting of 42 residues in two helices, has a flexible C-terminal (29). We performed a surface topography using castP analysis (30) in order to determine the binding pockets of these proteins, and the results are shown in Figure 4 and Figure 5. The embedded tables in these figures give the values of area and volume of each pocket in angstrom scale. The results reveal that in both cases the protein binding pockets are very small, thus these proteins very selectively bind to ligands or other proteins.

4. CONCLUSION

The intriguing observation of antimicrobial activities of neuropeptides and their structural similarities to AMPs is providing a stimulating concept of coevolution of neurohormones and peptides of the immune system. Much interest has been focused on development of therapeutic drugs from AMPs, but so far the many promises did not result into any major clinical application. NAMPs are scientifically interesting, but their dual functions complicate drug development. Surprisingly, amyloid-beta, might serve as an AMP. This observation suggests that amyloid-beta is not merely a component of disposed cellular waste, but also a contributor to the innate immune response.

5. REFERENCES

1. P Bulet, R Stocklin, L Menin: Anti-microbial peptides: from invertebrates to vertebrates. *Immunol Rev* 198, 169-184 (2004)

2. M Zasloff: Antimicrobial peptides of multicellular organisms. *Nature* 415, 389-395 (2002)

3. K Radek, R Gallo: Antimicrobial peptides: natural effectors of the innate immune system. *Semin Immunopathol* 29, 27-43 (2007)

4. Y Su, K Zhang, HJ Schluesener: Antimicrobial peptides in the brain. *Arch Immunol Ther Exp* 58, 365-377 (2010)

5. B Becher, I Bechmann, M Greter: Antigen presentation in autoimmunity and CNS inflammation: how T lymphocytes recognize the brain. *J Mol Med* 84, 532-543 (2006)

6. AR Simard, D Soulet, G Gowing, JP Julien, S Rivest: Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron* 49, 489-502 (2006)

7. RM Ransohoff, P Kivisakk, G Kidd: Three or more routes for leukocyte migration into the central nervous system. *Nat Rev Immunol* 3, 569-581 (2003)

8. J Falsig, J van Beek, C Hermann, M Leist: Molecular basis for detection of invading pathogens in the brain. *J Neurosci Res* 86, 1434-1447 (2008)

9. RE Hancock, HG Sahl: Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat Biotechnol* 24, 1551-1557 (2006)

10. M Zaiou: Multifunctional antimicrobial peptides: therapeutic targets in several human diseases. *J Mol Med* 85, 317-329 (2007)

11. L Zhang, TJ Falla: Host defense peptides for use as potential therapeutics. *Curr Opin Investig Drugs* 10, 164-171 (2009)

12. Y Lai, RL Gallo: AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. *Trends Immunol* 30, 131-141 (2009)

13. KA Brogden, JM Guthmiller, M Salzet, M Zasloff: The nervous system and innate immunity: the neuropeptide connection. *Nat Immunol* 6, 558-564 (2005)

14. MH Metz-Boutique, K Lugardon, Y Goumon, R Raffner, JM Strub, D Aunis: Antibacterial and antifungal peptides derived from chromogranins and proenkephalin-A. From structural to biological aspects. *Adv Exp Med Biol* 482, 299-315 (2000)

15. MH Metz-Boutique, Y Goumon, JM Strub, K Lugardon, D Aunis: Antimicrobial chromogranins and proenkephalin-A-derived peptides: Antibacterial and antifungal activities of chromogranins and proenkephalin-A-derived peptides. *Ann NY Acad Sci* 992, 168-178 (2003)

16. MH Metz-Boutigue, AE Kieffer, Y Goumon, D Aunis: Innate immunity: involvement of new neuropeptides. *Trends Microbiol* 11, 585-592 (2003)

17. M Salzet, A Tasiemski: Involvement of pro-enkephalin-derived peptides in immunity. *Dev Comp Immunol* 25, 177-185 (2001)

18. CJ Hansen, KK Burnell, KA Brogden: Antimicrobial activity of substance P and neuropeptide Y against laboratory strains of bacteria and oral microorganisms. *J Neuroimmunol* 177, 215-218 (2006)

19. J Li, X Xu, H Yu, H Yang, Z Huang, R Lai: Direct antimicrobial activities of PR-bombesin. *Life Sci* 78, 1953-1956 (2006)

20. KL Brown, RE Hancock: Cationic host defense (antimicrobial) peptides. *Curr Opin Immunol* 18, 24-30 (2006)

21. SJ Soscia, JE Kirby, KJ Washicosky, SM Tucker, M Ingelsson, B Hyman, MA Burton, LE Goldstein, S Duong, RE Tanzi, RD Moir: The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. *PLoS One* 5, e9505 (2010)

22. C Tukei, RP Wilson, JH Nishimori, M Pezeshki, BA Chromy, AJ Baumler: Responses to amyloids of microbial and host origin are mediated through toll-like receptors 2. *Cell Host Microbe* 6, 45-53 (2009)

23. AM Hartz, DS Miller, B Bauer: Restoring blood-brain barrier P-glycoprotein reduces brain amyloid-beta in a mouse model of Alzheimer's disease. *Mol Pharmacol* 77, 715-723 (2010)

24. N Mookherjee, KL Brown, DME Bowdish, S Doria, R Falsafi, K Hokamp, FM Roche, R Mu, GH Doho, J Pistolic, JP Powers, J Bryan, FS Brinkman, RE Hancock: Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37. *J Immunol* 176, 2455-2464 (2006)

25. H Jang, FT Arce, R Capone, S Ramachandran, R Lal, R Nussinov: Antimicrobial protegrin-1 forms amyloid-like fibrils with rapid kinetics suggesting a functional link. *Biophys J* 100, 1775-1783 (2011)

26. A Nijnik, RE Hancock: The roles of cathelicidin LL-37 in immune defences and novel clinical applications. *Curr Opin Hematol* 16, 41-47 (2009)

27. K Hase, M Murakami, M Iimura, SP Cole, Y Horibe, T Ohtake, M Obonyo, RL Gallo, L Eckmann, MF Kagnoff: Expression of LL-37 by human gastric epithelial cells as a potential host defense mechanism against Helicobacter pylori. *Gastroenterology* 125, 1613-1625 (2003)

28. G Wang: Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. *J Biol Chem* 283, 32637-32643 (2008)

29. O Crescenzi, S Tomaselli, R Guerrini, S Salvadori, AM D´Ursi, PA Temussi, D Picone: Solution structure of the Alzheimer amyloid beta-peptide (1-42) in an apolar microenvironment. Similarity with a virus fusion domain.

Eur J Biochem 269, 5642-5648 (2002)

30. J Liang, H Edelsbrunner, C Woodward: Anatomy of protein pockets and cavities: measurement of binding site geometry and implications for ligand design. *Protein Sci* 7, 1884-1897 (1998)

Abbreviations: AMPs: antimicrobial peptides; APP: amyloid precursor protein; BBB: blood-brain barrier; BRPs: bombesin-related peptides family; CNS: central nervous system; DCD: dermcidin; GI: gastrointestinal; LL-37: human cathelicidin; NAMPs: neuro-antimicrobial peptides; NK1: neurokinin-1; NPY: neuropeptide Y; PAMPs: pathogen-associated molecular patterns; PEA: proenkephalin A; SP: substance P; TLR: toll-like receptor

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