

Preface

In the reviews “The brain immune system: chemistry and biology of the signal molecules” as well as “Concepts of neuroendocrine cardiology and neuroendocrine immunology”, (1, 2) I have summarized the results of 45 years of laboratory findings on the discovery of a new hormonal system produced by human and animal neurosecretory cells of the hypothalamus. Discovery of neuroendocrine immune system of the brain (concept that the brain is an immune organ) (2) opens a new page in our understanding of the molecular mechanisms of the immune system regulation as a whole and immune defense of the brain itself. The proline-rich polypeptides are a new type of brain cytokines (3,4). One of them, galarmin (PRP-1), not only participates in the immune defense of the brain and the organism, but also in genesis, differentiation, proliferation, and mobilization of bone marrow progenitors. Signal molecules of the neuroendocrine immune system of the brain, galarmin, Gx-NH₂, and others contain 10–15 amino acids residues and possess wide antibacterial, anti-tumor, hematopoietic, and anti-neurodegenerative properties. Professor Abel Lajtha wrote “The importance of Galoyan’s concept is that hypothalamic peptides and cytokines, secreted by NSO and NPV, act not only as a component of the endocrine system, but also as modulators of the immune system and what is of great practical importance is the system can be neuroprotective and strong antibacterial”(5).

We have isolated a family of neuropeptides with cardiac activity and their precursor protein, as specific regulators of cardiac blood vessels and cardiovascular system from the human and animal hypothalamus (3). Moreover, in 1967, I found that the heart is an endocrine organ, the nerve cells of which (the atria) produce and secrete hormones of protein nature into the blood. They were also the regulators of cardiac blood flow and catecholamine biosynthesis in the atria and ventricles of the heart activity of the neurons. The atrial ganglionic cells exhibit a fine reactivity towards the hypothalamus cardioactive hormones through an increase in neurosecretion and the transition of products to the atrial axons (3).

The concept was established “The functional system of neurosecretory hypothalamus, the endocrine heart”.

There is a functional interaction between the neuroendocrine immune system of the hypothalamus and endocrine heart. Hypothalamic cardioactive neurohormone C stimulates biosynthesis of the gomori positive structures and produces positive

granule synthesis in atrial neurons and release of these granules through the neural termini. Later, we discovered neuroendocrine immune system of the brain, and the signal molecules of which proline-rich peptides produced by neuroendocrine cells of hypothalamus proved to be the regulators of immune system, and bone marrow function.

The results of such investigations led us to the foundation of two important trends of neurobiology: neuroendocrine cardiology and neuroendocrine immunology.

The data we obtained proved that one of the proline-rich peptides, galarmin, is a strong antibacterial (“non-specific” agent). Antibacterial properties of galarmin were tested on the following species of bacteria; *Salmonella typhumurium*, *Salmonella cholerae suis*, *Salmonella typhi*, *Escherichia coli*, *Shigella Flexneri*, *Pseudomonas aeruginosa*, *Shigella Sonnei*, *Staphylococcus aureus*, *Streptococcus pneumoniae* (4), *Bacillus anthracis* (6), *Clostridium perfringens* as well as *Mycobacterium tuberculosis*, *Methicillin-resistant S.aureus* etc. (see chapter 5–8).

Female, pathogen-free lines of mice, i.e. BALB/c, C57BL/61, C₃H/Hej, CBAxC57BL/6, F₁ age 5–8 weeks were used in the experiments.

Experimental data suggests that PRPs, mainly galarmin, Gx-NH₂, and d-15 Galarmin analogues are effective antibacterial drugs against *Bacillus anthracis*; the etiological agent of anthrax, which is a gram-positive, aerobic, spore forming, rod-shaped bacterium. *B.anthraxis* development in macrophages leads to their destruction during which the bacillus enters the blood stream and starts to replicate to large quantities for long periods. This data indicates the availability of a strong defensive reserve in the brain to fight against infections.

Epidemiological data indicate that *S. aureus*, particularly methicillin-resistant strain of *S.aureus* (MRSA), are responsible for the most of complicated cases of Staphylococcus infection and are increasingly implicated as a cause of nosocomial and community associated infections worldwide.

C. perfringens (anaerobic infection) is the causative agent of various diseases including gas gangrene and food poisoning. The bacterium produces numerous extracellular toxins (7,8). *C. perfringens* strain 13, the Virs/Vir R-two component system, is involved in the coordinated regulation of production of several toxins; the alpha toxin (Plc), theta toxin (Pro A), and the kappa-toxin (col A). *C. perfringens* strain 13 and its derivatives were grown under anaerobic conditions. Antimicrobial agents used in this study were as follows: cefoxitin, clindomycin hcl or clindomycin po4, metronidazole, metronidazole for iv injection, potassium penicillin G, penicillin sodium, rifampin, and tetracycline hci. A dose of penicillin G is 198–1500 mg/kg, of clindomycin. Metronidazole and rifampin doses are 8–12 mg/kg, clindomycin PO4 is 2.1 and 8.6 mg/kg, and metronidazole is 19 and 75 mg/kg. Shimizu T. and colleagues (9) described the whole genomic sequence of strain 13. The genes unique to *C. perfringens* are those encoding myoinositol catabolism proteins and α and β galactosidases, three extracytoplasmic function (ECF) type sigma factors, α mannosidase components for V type sodium ATP synthase, selenocysteine synthase and various virulence associated proteins. Gas gangrene caused by *C. perfringens* is a fulminant infection, which expedites progress to profound shock and death without

treatment. *C. perfringens* is more likely to be resistant to clindamycin, chloramphenicol, and tetracycline. In addition, the antibiotic resistance to penicillin, tetracycline, cephalosporin, clindamycin, and metronidazole was reported among clinical isolates of *C. perfringens* (8). Prophylactic serotherapy or antibiotic therapy is ineffective and in this case, high doses of antibiotics such as 20, 50, 75 mg/kg up to 86 mg/kg every 4 hours are being used. However, incomparable, small amounts of galarmin (micrograms range) and its analogues are used for the treatment of the multiple infectious diseases including gas gangrene caused by *C. perfringens*.

Huge experimental data obtained recently indicate the PRPs, namely galarmin, to be strong “non-specific” drug for the prophylaxis and treatment of anthrax, gas gangrene, tuberculosis, MRSA (septic infections), and myocardium infarctions etc. The physiology of *Mycobacterium tuberculosis* is highly aerobic and requires high levels of oxygen, as it is primarily a pathogen of the mammalian respiratory system. *M. tuberculosis* does not retain any bacteriological strain due to high lipid content in its wall and thus it is neither gram positive, nor gram negative, but classified as acid fast gram-positive bacteria. Mycobacterium outbreaks are often caused by hyper virulent strains of *M. tuberculosis*. The genome of H37Rv strain was published in 1948. The size of it is 4 million base pairs with 3959 genes. Within the genome there are also 6 pseudogenes along with 250 genes involved in fatty acid metabolism. Usually, the treatment is given for six to nine months, according to a therapy regimen consisting of two months treatment with isoniazid, rifampin, pyrazinamide, ethambutol or streptomycin until the drug sensitivity is known.

Fundamental investigations into the mechanisms of galarmin, Gx-NH₂, and analogues antibacterial actions indicate that they are different from other known antibacterial and/or proline-rich polypeptides such as RP-39, RP-26, etc. Galarmin participates in dose-related manner in the formation of HO• radicals in fenton-like reactions and inhibits Cu II dichloride catalyzed H₂O₂ decomposition, thus preventing formation of HO• and HOO• radicals (10). This peptide also manifests antiradical activity towards 2,2-diphenyl 1-picrylhydrazyl radicals, depending on the existence of phenolic OH group in tyrosine residue at the end of the molecule (11).

It was established that galarmin and Gx-NH₂ in low concentrations are scavengers of HO• radicals (10,11), whereas high concentrations promote HO• radicals formation in fenton-like reaction (10). The data was obtained with galarmin's participation in oxidative bursts in dose-dependent manner in neutrophils, monocytes, and macrophages in normal and inflammatory diseases, such as Bechet's and Familial Mediterranean Fever (FMF) diseases (12,13).

Being a mediator between the brain neuroendocrine system and bone marrow, these cytokines take part in genesis, differentiation, and proliferation of the stem cells in the bone marrow. Experimental results prove the discovery of the novel class of powerful compounds against both aerobic and anaerobic infections that will have its impact in preventing biological terrorism.

Thus, the role of brain immunomodulators and cytokines in the complicated metabolic and neuroendocrine processes should be discussed in the neuroendocrine-immune network context at health and disease statuses. The elucidation of the

molecular mechanisms of PRP-1's numerous effects on a number of aerobic or anaerobic infections represents very big interest.

The present book contains description of all the main components of the immune system (cytokines, chemokines, new immunomodulators and a new cytokine, proline-rich polypeptides) indicating mainly the antibacterial properties of PRPs, particularly of galamin.

The importance of new brain immunomodulators and cytokines (mostly produced by neuroendocrine cells of the hypothalamus) in a variety of adaptive reactions of the organism, infectious diseases, cancer, neurodegenerative, etc. should be emphasized. However, in this book I focus on the antibacterial properties, mainly proline-rich polypeptides, thoroughly discussing the most common biochemical, neurohormonal, and genetic mechanisms of their influence on the most severe aerobic and anaerobic infections.

Professor Armen Galoyan, M.D., Ph.D.