

MEETING ABSTRACTS

THE FIRST INTERNATIONAL MEETING OF ASIA MINOR BRANCH
OF THE INTERNATIONAL NEUROPEPTIDE SOCIETY
21-25 MAY, 2001, ANTALYA, TURKEY

On 21-25 May, 2001 in Antalya, Turkey, the First International Meeting of Asia Minor Branch of the International Neuropeptide Society (INPS) was held in an atmosphere combining both science and friendship. Professor Dr Neşe Tunçel from Osmangazi University Medical School, Eskişehir, Turkey, functioning as Chairman, did a superb job of organizing the Meeting. The Antalya INPS Meeting emphasized the increasing significance of neuropeptides and related molecules for the regulation of neural, immune and endocrine responses in physiological and pathological conditions. The Meeting was attended by renowned scientists, contributing to the recent advance in the study of neuropeptides and neurotrophins, including S.I. Said from USA, the discoverer of vasoactive intestinal peptide (VIP), S.M. McCann, Doina Ganea, Pan Weihong, I. Rubinstein, and W.A. Banks from USA, L. Aloe from Rome, Italy, Rosa P. Gomariz from Madrid, Spain, and R. Ekman from Mölndal, Sweden. Their state-of-the-art lectures, as well as those presented by other participants, encouraged and facilitated creative discussions. Altogether, this cultivated much a new thinking about the therapeutic potentials of various neuropeptides and neurotrophins, particularly, VIP and nerve growth factor, respectively. All these events were organized in the atmosphere of a scientific family gathering in Falez Hotel placed on a Mediterranean sea bay of the beautiful Antalya. Here, we present the opening speech of Dr Tunçel and a set of selective abstracts of the Antalya INPS Meeting 2001.

Opening Speech of Chairman

*If you have an apple and I have an apple, and we exchange apples, each of us will have an apple.
However, if you have one idea and I have another idea, and we exchange ideas, each of us will have two ideas.
Paraphrased from Albert Einstein*

Dear Colleagues,

It is with great honor and pleasure that I welcome you to the First International Meeting of Asia Minor Branch of The International Neuropeptide Society (INPS) in Antalya, Turkey. The program of the Meeting focuses on Neuropeptides and Immune cells: Neuropeptides in Regulation of the Immune Response.

The recent earthquake and present economic crisis in Turkey have had a dramatic effect on this meeting. However, we did not give up and pursued its organization. Hence the First Meeting of Asia Minor Branch of INPS could be realized. This first meeting is a very small one now, but we believe that it will continuously grow, like an embryo. On behalf of the Organizing Committee I would like to thank all the eminent lecturers and participants that attending the Meeting. The Meeting's major topic will provide an overview of important research lines related to the modulation of a wide range of immunologic and inflammatory responses by neuropeptides

and related molecules. And, will stimulate the free expression and exchange of ideas, in an Einsteinian way. Recent research on neuropeptides continues to generate much interest among the investigators in the field of biomedical science. Neuropeptides play pivotal roles as mediators in neurons, endocrine cells and immune cells, and are thus critically involved in the regulation of cross-talk between these cells. A clearer discernment of neuro-immune-endocrine communication is dramatically altering our understanding of physiology and pathology. And, it may also profoundly affect the therapy of a wide variety of diseases.

Finally, the Organizing Committee's members express their sincere thanks to the individuals and organizations whose support made the Antalya INPS Meeting possible.

*Dr Neşe Tunçel
Chairman of the Meeting*

CONTROL OF THE RELEASE OF LEPTIN, TUMOR NECROSIS FACTOR-ALPHA AND NITRIC OXIDE BY THE CENTRAL NERVOUS SYSTEM

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Evidence is accumulating that the release of cytokines and nitric oxide (NO) is primarily controlled by the central nervous system, although there is also local control exercised in the tissues that produce these powerful mediators. There is a circadian rhythm of leptin release in man and rat with peak values at 1:30 a.m. which appears to be partly controlled by secretion of prolactin since prolactin stimulates leptin release and the inhibitor of prolactin secretion bromocryptine lowers it. The levels of nitrate/nitrite (NO_3/NO_2) in plasma reflects the production of NO throughout the body and the circadian rhythm of NO_3/NO_2 parallels that of leptin suggesting that leptin may be mediating the rhythm of NO production. Indeed, incubation of leptin with epididymal fat pads induces the release of NO. Anesthesia causes a decline in both plasma leptin and NO_3/NO_2 providing further evidence of neural control of both substances. Bacterial lipopolysaccharide (LPS), which causes responses in the body that mimic those of infection causes a gradual release of leptin, that can be inhibited

by bromocryptine or dexamethasone, and by inhibition of α -adrenergic and β -adrenergic inhibitory control over leptin release. Tumor necrosis factor (TNF)- α responds rapidly to LPS, and in contrast to leptin, although this response is blocked by anesthesia and a β -adrenergic agonist, there appears to be a stimulatory, instead of inhibitory α -adrenergic control involved in the response. Not only is TNF- α release stimulated by LPS, but it is remarkably stress-responsive showing a 400-fold increase to the minor procedure of external jugular catheterization. This response is delayed by the anesthesia during the surgery. Stress induces a dramatic, rapid decline in plasma NO_3/NO_2 , not related to the slight decline in plasma leptin that may be caused by a neurally mediated inhibition of NOS. That the central control is supplemented by local control is shown by the fact that incubation of epididymal fat pads with leptin not only increases NO production, but at lower concentrations activates TNF- α release. This work was supported by NIH grant MH51853.

IMMUNOBIOLOGY OF VASOACTIVE INTESTINAL PEPTIDE AND PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE: ROLE IN INFLAMMATORY DISEASES

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Vasoactive intestinal peptide (VIP) and the structurally related peptide, the pituitary adenylate cyclase-activating polypeptide (PACAP), are two multifunctional and pleiotropic mediators shared by nervous, endocrine and immune systems. In the immune system, both peptides are produced by the same lymphocyte subpopulations exerting their actions through a family of G-protein coupled receptors named VPAC1, VPAC2 and PAC1. These peptides have an important role modulating both the immune homeostasis and the inflammatory response in normal and pathological conditions. Whereas the inflammatory response is essential for the ultimate elimination of antigens, its duration and intensity have to be controlled to avoid extensive tissue damage. An insufficient inflammatory response could compromise the survival of the organism, but an excessive response could develop into acute and chronic inflammatory diseases. The balance of the proinflammatory and antiinflammatory cytokine network is determinant in the final outcome of the inflammatory response. In the last four years, our group has focused on the role of VIP, PACAP, and their agonists as antiinflammatory factors

in several inflammatory disorders such endotoxic shock and rheumatoid arthritis. Our results indicate that both peptides and the VPAC1 agonist attenuate the deleterious consequences of septic shock by inhibiting the production of proinflammatory cytokines and radicals such as TNF- α , IL-6, and IL-12 and nitric oxide, and stimulating the production of antiinflammatory cytokines such as IL-10 in macrophages. In addition, treatment with VIP, PACAP and VPAC1 agonist result in a delayed onset, lower incidence and decrease severity of an experimental model of arthritis. This preventive effect is mediated through the inhibition of both proinflammatory and autoimmune components of the disease. The antiinflammatory action is also mediated by downregulating the expression and production of proinflammatory cytokines, inducible nitric oxide synthase and chemokines, and by upregulating the expression of the antiinflammatory agents IL-10 and IL-1Ra. The autoimmune component is abolished by driving the response toward a Th2 one. Our results suggest VIP, PACAP and their analogs as very attractive candidates for the treatment of acute and chronic inflammatory diseases.

INHIBITION OF ENDOTOXIN-INDUCED MACROPHAGE CHEMOKINE PRODUCTION BY VASOACTIVE INTESTINAL PEPTIDE AND PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE *IN VITRO* AND *IN VIVO*

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Inflammatory chemokines recruit various populations of immune cells which initiate and maintain the inflammatory response against foreign antigens. Although such a response is necessary for the elimination of the antigen, the inflammation has to be eventually resolved in a healthy organism. Peptides such as vasoactive intestinal peptide (VIP) and pituitary adenylyl cyclase-activating polypeptide (PACAP), released following antigenic stimulation, contribute to the termination of an inflammatory response primarily by inhibiting the production of proinflammatory cytokines. Here we investigated the effects of VIP and PACAP on chemokine production. We report that VIP and PACAP inhibit the expression of the macrophage-derived CXC chemokines MIP-2 and KC (IL-8), and of the CC chemokines MIP-1 α , MIP-1 β , MCP-1, and

RANTES *in vivo* and *in vitro*. The decrease of chemokine gene expression correlates with an inhibitory effect of VIP/PACAP on NF κ B binding and transactivating activity. The VIP/PACAP inhibition of both chemokine production and of NF κ B binding and transactivating activity is mediated through the specific receptor VPAC1, and involves both cAMP-dependent and -independent intracellular pathways. In an *in vivo* model of acute peritonitis, the inhibition of chemokine production by VIP/PACAP leads to a significant reduction in the recruitment of PMNs, macrophages and lymphocytes into the peritoneal cavity. These findings support the proposed role of VIP and PACAP as key endogenous antiinflammatory agents, and describe a novel mechanism, i.e., the inhibition of the production of macrophage-derived chemokines.

VIP AND PACAP ENHANCE THE *IN VIVO* GENERATION OF MEMORY TH2 CELLS BY INHIBITING PERIPHERAL DELETION OF ANTIGEN-SPECIFIC EFFECTORS

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During a primary immune response, resting naive CD4 T cells specific for the antigen proliferate and differentiate into large, activated effector cells capable to rapidly produce large amounts of cytokines upon restimulation. At this stage, the effector T cells are susceptible to antigen-induced cell death (AICD), mediated primarily through FasL/Fas interactions. A low percentage of the activated effector T cells are however protected from apoptosis and differentiate into long-lived memory T cells. Therefore, during an immune response, mechanisms must operate not only to destroy not longer needed or even potentially damaging T cells, but also to allow the survival of a small number of activated T cells. At the present time little is known about the factors and the mechanisms that regulate the shift from an apoptosis-resistant to an apoptosis-sensitive phenotype and *vice versa*. Vasoactive intestinal peptide (VIP) and the structurally related peptide, the pituitary adenylyl cyclase-activating

polypeptide (PACAP), are two peptides synthesized by lymphocytes that elicit a broad spectrum of biological functions, including actions on innate and adaptive immunity. Recently, VIP and PACAP were shown to also inhibit AICD in peripheral CD4 T cells through the downregulation of FasL expression. In view of these findings, VIP and PACAP are reasonable candidates for the generation of memory and specific effector T cells following antigen stimulation. To test this hypothesis, we analyzed the effects of VIP and PACAP in various models for effector and memory T cells. Our data demonstrate that both neuropeptides differentially promote the *in vivo* effector function and memory phenotype of Th2, but not Th1 cells, by preferentially inhibiting the clonal deletion of Th2 cells. To our knowledge, this is the first report describing the role of a peptide present in the lymphoid microenvironment on the generation and maintenance of long-lived memory T cells.

TH2 LYMPHOCYTES SECRETE FUNCTIONAL VASOACTIVE INTESTINAL PEPTIDE UPON ANTIGEN STIMULATION

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In addition to the peptidergic innervation, immune cells may also represent a source for vasoactive intestinal peptide (VIP) in the lymphoid organs. Previous studies reported increased mRNA^{VIP} and protein expression in mitogen-stimulated B and T lymphocytes. To determine whether specific T cell subsets are responsible for VIP production, we derived Th1 and Th2 effector cell lines from T-cell receptor transgenic mice. Th1 and Th2 cells were stimulated with the specific (pigeon cytochrome C peptide) or nonspecific (ovalbumin) antigen presented by MHC class II compatible antigen-presenting cells. Upon stimulation with the specific antigen, Th2, but not Th1 cells express mRNA^{VIP} and intracellular VIP protein, as de-

termined by Northern blots and FACS analysis. Supernatants harvested from antigen-stimulated Th2 cells contain secreted VIP, as determined by ELISA, and induce cAMP in HEK293 cells transfected with the specific VIP/PACAP receptor VPAC1. These results confirm that Th2, but not Th1 cells, express and secrete functional VIP following specific antigen stimulation. The release of VIP within the lymphoid microenvironment following antigenic stimulation provides a physiological basis for the immunoregulatory effects of VIP on neighboring immune cells, such as downregulation of macrophage activation, and effects on lymphocyte migration, antigen-induced T cell apoptosis, and T cell differentiation.

EFFECTS OF SEPSIS ON MAST CELLS IN RAT DURA MATER : INFLUENCE OF L-NAME AND VASOACTIVE INTESTINAL PEPTIDE

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The influence of lipopolysaccharide (LPS)-induced sepsis on the various mast cell phenotypes of rat dura mater were examined both by immunohistochemical and biochemical methods. Three different populations of mast cells were identified in control rats: connective tissue type mast cells (CTMC) which contain rat mast cell protease1 (RMCP1), histamine, serotonin and heparin, mucosal type mast cells (MMC) which contain RMCP2, histamine and serotonin, and intermediate type which contains both RMCP1 and RMCP2 and probably various proportions of amines and heparin. LPS (25 mg/kg⁻¹ i.p.) caused changes in the proportions of the various types of mast cells. The number of MMC and intermediate type mast cells significantly increased and the number of mast cells immunopositive for both heparin and serotonin significantly de-

creased. Biochemical analysis showed that the histamine concentration of dura increased while its serotonin concentration decreased. While vasoactive intestinal peptide (VIP) (25 ng/kg⁻¹ i.p.) appears to potentiate LPS effects on dura mater mast cells, nonselective inhibition of nitric oxide (NO) synthase by Ng-nitro-L-arginine methyl ester (L-NAME) (30 mg/kg⁻¹ i.p.) did not influence sepsis-induced mast cell changes. Altogether, these findings suggest that mast cells of dura mater may play a role in brain protection during sepsis. They also confirm that mast cell reaction to sepsis can vary according to the organ considered since, in the dura mater, the reactions to NO and VIP can be compared to what we found with other techniques in liver but not in kidney.

NERVE GROWTH FACTOR AND NEUROIMMUNE RESPONSES: BASIC AND CLINICAL OBSERVATIONS

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Nerve growth factor (NGF), first identified for its activities in promoting the growth and differentiation of sensory and sympathetic neurons, exerts a modulatory role on neuroimmune-endocrine functions playing an important role in the regulation of homeodynamic processes. NGF-responsive cells belonging to the immune and to the neuroendocrine systems have been identified and characterized. NGF is involved in a number of autoimmune disorders, such as multiple sclerosis, rheumatoid arthritis, and lupus erythematosus. Moreover, the changes in NGF levels are associated with an accumulation of mast cells (MC), cells that are implicated in neuroimmune interaction and tissue inflammation. Because MC are able to respond not only to the action of NGF, but also are able to synthesize, store, and release NGF, it is highly possible that alterations in normal MC distribution and behavior may be linked to maladaptive

neuroimmune tissue responses or to promote reparative actions of damaged cells. Since autoimmune diseases are characterized by an abnormal activation of cells of the immune, nervous and endocrine system and since NGF appears to play a crucial role on some specific cell population of these three systems, the major aim of our studies in the last ten years has been to identify effects, functions and mechanisms linking the altered constitutive levels of NGF and the development of certain autoimmune inflammatory disorders. Previous and ongoing studies supporting a role of NGF in these disorders will be presented and discussed (1-4).

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NEUROIMMUNOLOGICAL DEFICITS INDUCED BY *SCHISTOSOMA MANSONI* INFECTION IN A MOUSE MODEL

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Schistosoma mansoni infection (schistosomiasis) is a parasitic disease caused by several species of schistosome worms, affecting several million of young and adult people mainly in Africa, South America, Middle East, Southeast Asia and China. The key pathological event in this disease is the formation of granulomas around schistosome eggs in the liver, intestine and central nervous system. Granulomas, which are a particular form of inflammation, are mainly caused by the host's reaction to the parasitic infection and are characterized by an aggregation of macrophages, lymphocytes and eosinophils around schistosome eggs. The presence of granulomas in the liver is associated with local fibrosis and venous hypertension, which seems to be the main cause of morbidity and mortality in human and rodent schistosomiasis. This inflam-

matory disease is also characterized by the release of several biologically active compounds including nerve growth factor and cytokines, such as IL-2, IL-5, tumor necrosis factor-alpha (TNF- α). This latter cytokine appears to be functionally relevant, since injections of TNF- α antibody reduce the development and the formation of granulomas. The presence of brain granulomas is associated with both peripheral and central neurological dysfunctions, thus the utilization of animal behavior may be extremely useful for studying specific sets of behavioral and neuroimmunological deficits. These data will be presented and discussed (1,2).

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MASS SPECTROMETRY IN PSYCHONEUROIMMUNOLOGY: LYMPHOCYTES REFLECTING PSYCHIATRIC DISORDERS

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Understanding the biology of the human brain is a challenging task. There is an ongoing accumulation of data supporting a molecular interaction between the central nervous system and the immune system under normal and diseased conditions, reflected in protein/peptide alterations in circulating cells of the immune system. Psychiatric disturbances and even disorders as depression, schizophrenia and dementia might cause these chemical modifications. Recent advances in the field of mass spectrometry allow for both molecular weight and structure analysis of low-abundant biomolecules. The technical advances in instrumentation and methodologies as modern proteomic and peptidomic tools have made it possible to determine molecular masses of macromolecules, to sequence proteins/peptides and to determine posttranslational modifications of proteins. We have investigate whether cytoplasmatic and nuclear extracts of human peripheral blood

lymphocytes contain the neuropeptide AVP, claimed to be of importance for memory functions, in samples from healthy controls and patients diagnosed as depressed. Such knowledge has direct implications not only for research on normal brain function but also provides a basis for the development of new diagnostics and targeted treatments of these debilitating disorders. It is the first time as AVP, AVP-fragments (metabolites) and chemically modified AVP-forms have been demonstrated in HPLC-purified lymphocyte/nuclear extracts. This was performed by first using a HPLC - purification step, followed by a second immunoprecipitation step before identification by MALDI - TOF MS. The role of nuclear forms of AVP is still to be resolved, but may involve interactions with the transcription machinery through brain-derived neurotrophic factor. Finally we will address the use of a novel CE-MALDI and an on-line CE-FTICR interface to study peptide profiles from lymphocyte extracts.

NEUROTROPHINS, ADIPOKINES, AND IMMUNE CELLS IN HUMAN CARDIOVASCULAR PATHOLOGY: A NEW ROAD AHEAD

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Here we highlight available data of neuroimmune and adipo-immune mechanisms involved in the pathobiology of atherosclerotic cardiovascular disease and related disorders, such as metabolic syndrome (MS). While multiple growth factors/cytokines and also immune cells that are potential sources of neurotrophins are identified in atherosclerotic lesions, as well as an essential nonneuronal function of neurotrophins, particularly, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), are implicated in cardiovascular tissue development, the role of neurotrophins and of adipokines, adipose tissue-secreted cytokines, in atherosclerosis and MS has only recently emerged. We recently reported that (i) NGF vascular tissue levels were reduced and p75NGF receptor expression and mast cell number increased in human atherosclerotic coronary arteries compared with control specimens obtained from autopsy cases, while (ii) the respective inter-

nal mammary arteries revealed no atherosclerotic lesions, and also no difference in both NGF levels and mast cell number, and (iii) NGF, BDNF and interleukin-6 plasma levels were reduced in patients with MS compared with control subjects. Also, in MS patients, a positive correlation between the plasma leptin levels and the number of mast cells in biopsies from abdominal subcutaneous adipose tissue was found, implying the adipokine leptin as a potential mast cell growth factor. Altogether, these findings, taken in conjunction with data reported by other colleagues, are reviewed. We suggest that the neuroimmune/adipoimmune road proposed might be one among many roads which leads to atherosclerotic cardiovascular disease and related disorders. Reward is a matter of further collaborative work. Supported by grants from NATO/CNR, Rome, Italy Research Program.

NEUROIMMUNE PEPTIDE INTERACTIONS WITH THE BLOOD-BRAIN BARRIER

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Blood-borne cytokines have many effects on the central nervous system (CNS). We have described one mechanism by which these cytokines can affect the CNS: the ability of the blood-brain barrier (BBB) to directly transport selected cytokines into the CNS. Separate saturable systems exist for the interleukin-1 (IL-1) family (alpha, beta, and receptor antagonist), for tumor necrosis factor-alpha, for IL-6, and for some of the other cytokines. These systems have modest transport rates (K_i about 0.5 microl/g-min) with about 0.05-0.6 % of an intravenously injected dose entering each gram of brain. For example, IL-1alpha (IL-1 α) enters the poste-

rior division of the septum (PDS) at a very high rate. Human IL-1 α (huIL-1 α), which crosses the mouse BBB, impairs memory whether injected into the PDS or intravenously, although an intravenous dose 1000 times greater than the PDS dose is needed to achieve a given level of impairment. Blocking antibodies species-specific for huIL-1 α injected into the PDS of the mouse prevent about 70% of the impairment in memory induced by intravenous huIL-1 α . This shows that the PDS is a site of action of IL-1 α and that the transport of huIL-1 α across the BBB is one mechanism by which the blood-borne cytokine affects the CNS.

UPREGULATION OF THE TRANSPORT SYSTEM FOR TNF- α AT THE BLOOD-BRAIN BARRIER**P. Weihong and A. J. Kastin***V.A. Medical Center and Tulane University School of Medicine, New Orleans, LA, USA*

The transport system for cytokine tumor necrosis factor-alpha (TNF- α) at the blood-brain barrier (BBB) enables an enhanced yet saturable entry of TNF- α from blood to the central nervous system (CNS). This review focuses on the selective upregulation of the transport system for TNF- α at the BBB that is specific for the type of pathology, region, and time. The upregulation is reflected by increased CNS tissue uptake of radiolabeled TNF- α after intravenous injection in mice, and by inhibition of the increase with excess non-radiolabeled TNF- α . Spinal cord injury (SCI): upregulation of TNF- α uptake after thoracic transection is seen in the delayed phase of BBB disruption at the lumbar spinal cord. Thoracic

SCI by compression, however, has a longer lasting impact on TNF- α transport that involves thoracic and lumbar spinal cord, in contrast to the upregulation confined to the lumbar region in lumbar SCI by compression. Regardless, the uptake of TNF- α by spinal cord does not parallel BBB disruption measured by the leakage of radiolabeled albumin. Experimental autoimmune encephalomyelitis: The increase in the differential permeability to TNF- α seen in all CNS regions (brain and cervical, thoracic, and lumbar spinal cord) has a distinct time course and reversibility, and exogenous TNF- α has biphasic effects in modulating functional scores. The BBB, a dynamic, regulated barrier, is actively involved in disease processes.

CYTOPROTECTION BY VASOACTIVE INTESTINAL PEPTIDE AND PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE

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Considerable experimental evidence supports the view that vasoactive intestinal peptide (VIP) has a broad protective influence on various cells and tissues. This evidence has been gained from studies of cell injury in lung, heart muscle, and neuronal cells, and from survival experiments in animals subjected to endotoxin shock. Similar results have been obtained with the closely related pituitary adenylate cyclase-activating peptide (PACAP). Multiple mechanisms explain the cytoprotection afforded by VIP and PACAP. Among these are antiin-

flammatory actions, including inhibition of nuclear transcription factor NF- κ B activation and suppression of inflammatory cytokine expression and release. In addition, the peptides have antiapoptotic properties that promote cell survival. Both peptides thus offer the promise of novel therapeutic approaches in such conditions as acute lung injury (presenting as the acute respiratory distress syndrome or ARDS), myocardial ischemia, neuronal cell loss in stroke, head trauma, neurodegenerative diseases, as well as septic shock.

THE BIOLOGICAL EFFECTS OF ALPHA HELIX VASOACTIVE INTESTINAL PEPTIDE

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Vasoactive intestinal peptide (VIP) is a pleiotropic 28-amino acid amphipathic neuropeptide widely distributed in mammalian tissues. Unfortunately, the biological effects of the peptide *in vivo* are short-lived due, most likely, to its rapid degradation and inactivation. Previous studies showed that spontaneous interactions of VIP with organic solvents alter the conformation of the VIP molecule from random coil in an aqueous environment to alpha helix in organic solvents. The latter is optimal for ligand-receptor interactions and protects VIP from degradation. However, the relevance of this phenomenon to VIP bioactivity are uncertain. To this end, work from our laboratories showed that spontaneous interactions of VIP with conventional liposomes and sterically stabilized liposomes and micelles composed of biocompatible and biodegradable phospholipids amplify and prolong the bioactivity of VIP. This was evaluated by oral epithelial cell proliferation *in vitro* and changes in vasomotor tone and systemic

arterial pressure *in vivo*. The vasoactive effects of phospholipid-associated VIP were amplified in the presence of low concentrations of calmodulin. In addition, phospholipid-associated VIP abrogated vasoconstriction elicited by angiotensin II and phenylephrine. The bioactive effects of phospholipid-associated VIP, unlike those of aqueous VIP, were transduced by the L-arginine/nitric oxide biosynthetic pathway. Circular dichroism analysis of phospholipid-associated VIP revealed a significant increase in alpha helix content relative to aqueous VIP that was amplified by calmodulin and increasing temperature. Collectively, these data indicate that VIP interacts avidly with phospholipids. This process alters the molecular conformation of the peptide and amplifies its bioactivity. We propose that these distinct attributes could be used for peptide therapeutics in impotence, hypertension, sepsis, asthma and other disorders

DOES LEPTIN MODULATE IMMUNE AND ENDOCRINE RESPONSES TO LIPOPOLYSACCHARIDE-INDUCED ACUTE INFLAMMATION?

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In many studies it has been reported that leptin may play an important role not only in the regulation of food intake and body weight but can modify immune and endocrine response. The aim of this study was to estimate the effects of leptin administration on proinflammatory and antiinflammatory cytokines and pituitary, thyroid, adrenal and gonadal hormones in response to lipopolysaccharide (LPS)-induced acute inflammation in male Wistar-Kyoto rats. Cytokines concentrations were estimated using ELISA and hormones concentrations using RIA methods. LPS injection caused increasing tumor necrosis factor- α , interleukin (IL)-6, IL-10 concentrations ($p < 0.01$, $p < 0.01$, $p < 0.01$, respectively) as well as LPS lead

to decrease of T3, T4, testosterone, LH and prolactin ($p < 0.01$, $p < 0.01$, $p < 0.05$, $p < 0.01$, $p < 0.05$, respectively) and to increase of corticosterone ($p < 0.05$). After leptin administration we observed an increase in testosterone and corticosterone concentrations and decrease of T3 levels; however, we did not find differences in cytokine concentrations. We conclude that leptin modulates acute inflammation through intensity of LPS-induced corticosterone secretion and decreasing inhibiting role of LPS in testosterone secretion. Lack of changing in serum proinflammatory and antiinflammatory cytokines concentrations indicates that leptin modulates endocrine system through other mechanism during acute inflammation.

GENDER DIFFERENCES IN BLOOD-BRAIN BARRIER PERMEABILITY DURING PATHOLOGICAL CONDITIONS

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There are numerous reports showing that the brain function and metabolism are subject to sex differentiation. Sex related differences in the patterns of human electroencephalograms, evoked potentials, cerebral glucose metabolism and cerebral blood flow have been reported. On the other hand, most of the experiments on the blood-brain barrier (BBB) permeability has been induced in male animals. We have recently investigated the effects of pentylentetrazol induced seizure, adrenaline-induced acute hypertension and the effects of systemic hypoosmolar fluid loads on the BBB permeability in female and male rats (1-3). Adult male and female Wistar albino rats (weight 240-380 g) were examined in this study. Evans blue was used as an BBB tracer. Mean arterial blood pressure was recorded continuously during experiments. Epi-

leptic seizure was induced by pentylentetrazol (80 mg/kg i.v). Acute hypertension was induced adrenaline (40 µg/kg i.v). The plasma osmolarity was decreased by a single intraperitoneal injection of warm distilled water. Epileptic female rats were shown to have a large increase in BBB permeability in comparison to male rats in both hypoosmotic and epileptic conditions, while no significant difference on BBB breakdown was found between male and female rats in adrenaline-induced acute hypertension.

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IMPACT OF STRESS, GENDER AND MENSTRUAL CYCLE ON IMMUNE SYSTEM: POSSIBLE ROLE OF NITRIC OXIDE

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Stress is a factor found to be involved in the pathobiology of many diseases. Gender and menstrual cycle phases are other factors affecting the predisposition of individuals for certain diseases. Results from animal and human studies suggest that the distribution of immune system cells may change at different phases of the menstrual cycle. Acute mental stress also alters immune variables. Although the increase in the number of natural killer (NK) cells is the most consistent finding among the immune variables, there are controversies for the other lymphocyte groups. Nitric oxide (NO) as an immune mediator has an unsettled role whether it causes the redistribution of the immune cells, or is an end product of lymphocyte activation. This study was planned to investigate the effect of mental stress on lymphocyte subtypes and the role of NO in men and women, and at different phases of the menstrual cycle. For this purpose, healthy women during the follicular and luteal phases (n=10) and men (n=10) underwent Stroop color-word interference and cold pressor tests. The

immune system responses before and after the tests were determined flowcytometrically. Menstrual cycle phase was ascertained by plasma estrogen and progesterone levels. Stress response was evaluated by blood pressure and heart rate measurements throughout the tests, and plasma cortisol and urinary metanephrine and vanillylmandelic acid measurements before and after the tests. Plasma and urinary NO determinations were performed before and after the test was completed. All the results were analysed with the appropriate statistical methods. The luteal phase differed from the other groups due to the presence of suppressed immune response to acute stress, including decreased CD4/CD8 ratio and NK cell percentage. On the other hand, acute stress caused a shift from cellular to humoral immunity in men. As indicated by these results, individual reaction towards stress is affected by gender and menstrual cycle phase. Nitric oxide appears to be a possible effector molecule mediating these differences.