In memory to D. S. Sarkisov

From Neuroendocrinology to Neuroimmunoendocrinology

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* 131, No. 1, pp. 22-32, January, 2001
Original article submitted December 13, 2000

Neuroimmunoendocrinology, a new integral biomedical discipline born at the interface of the two last centuries, unites and coordinates studies of the interactions between the major regulatory systems – nervous, endocrine, and immune. In this review we discuss the reasons for distinguishing neuroimmunoendocrinology as a special discipline and sum up the development of its modern concepts.

Era of Neuroendocrinology

Neuroendocrinology arose from discoveries made at the beginning and in the middle of the 20th century, when hypothalamic neurons were shown to secrete peptide neurohormones and retain their peculiar organization and impulse activity. It was found that large neurons of the preoptic nucleus of fish hypothalamus (corresponding to the supraoptic and paraventricular hypothalamic nuclei in mammals) synthesize nonapeptides (oxytocin and vasopressin homologues), transport them along axons into the posterior pituitary, and then store and release these molecules into the circulation [90,91]. This latter function united the hypothalamic neurons with secretory cells of endocrine glands, and therefore the phenomenon was denoted as neurosecretion [9,24,92,93].

It was than found that peptide neurohormones are also secreted by small hypothalamic neurons regulating the endocrine functions of the anterior pituitary by producing stimulatory (releasing hormones or liberins) and inhibitory (statins) neurohormones delivered into the anterior pituitary through the pituitary portal vessels [81,89].

Finally, when receptors for hormones of peripheral endocrine glands were detected on membranes of hypothalamic secretory neurons by analogy with pituitary cells, the mechanisms of hypothalamic regulation of endocrine functions became clear. As was previously demonstrated for the pituitary, they are based on the feedback principle [103]: hypothalamic and pituitary cells receive information on hormonal activity at the periphery and correct hormone homeostasis by releasing stimulatory or inhibitory hormones into the portal vessels of the pituitary.

These outstanding discoveries became the start point for neuroendocrinology, a new science for that time [1,94,103]. Born at the interface of neurobiology and endocrinology, neuroendocrinology provided us with new concepts on the mechanisms underlying the regulation of the key homeostatic functions. It was found that similar mechanisms underlie the regulatory effects of the nervous and endocrine systems: secretion of a regulatory peptide is determined by the extent and direction of deviations in the hormonal homeostasis.

Further development of neuroendocrinology brought new bright discoveries, which first surprised the scientists. It was found that peptide neurohormones are secreted not only in the hypothalamic region of the brain, but also in the entire central and peripheral nervous system [67,102]. However, we should like to emphasize that peptides synthesized by neurons other than hypothalamic are not released into local (portal) or systemic blood flow, but only locally into intercellular spaces and their effects are paracrine or mediated by secretory-motor innervation. Therefore, the concept of neurosecretion is first referred only to hypothalamic neurons releasing neuropeptides into systemic liquid media (blood and liquor) and exerting distant effects.

Due to the development of immunocytochemical methods, neuropeptides were revealed in the autonomic nerve endings innervating the intestine [60]. Detec-

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0007-4888/01/1311-0015$25.00

tion of peptide hormones secreted by the pituitary and peripheral endocrine glands, including the endocrine cells of the intestine, skin, and other organs [61,102], was still more unexpected. These facts were difficult to explain by the traditional concepts of biology. More general biological (in particular, genetic) approaches were needed. The concept of the diffuse endocrine system [53, 54] and APUD concept developed by A. G. E. Pearse [79,80] were rather fruitful. The latter concept is well known, and there is no need to discuss it in detail. We are interested now only in its principal assumptions, which can shed light on the interpretation of the above-mentioned facts.

According to this concept, which is best of all applicable to adrenal chromaffine cells and paraganglia, embryonic cells from which neurons are determined initially possess a genetic program of synthesizing both mediators and hormonal peptides. Depending on the expression of their gene loci, they are differentiated into classical neurons or into neuroendocrine and endocrine cells.

Scientists developing this idea regard endocrinology as a private case in neuroendocrinology [59], and in classification of the nervous system elements distinguish the somatic and autonomic nervous system and endocrine system as its components. This concept helps us understand why peptides are present in many cerebral neurons, though they were believed to be synthesized only by endocrine glands. From this viewpoint it is obvious that the neuroendocrine center no longer includes only the brain, its hypothalamic area in particular, and can also include peripheral autonomic ganglia, final points in the feedback with circulating hormones, which leads to secretion of the appropriate neuropeptides [8].

Which is the role of peptide in nervous cells? On the one hand, neuropeptides meet all the requirements to neurotransmitters: in surviving brain sections they are released from nerve endings in response to depolarization; this process is calcium-dependent and is suppressed in the presence of magnesium ions; after centrifugation neuropeptides are detected in synaptosome fractions, they modify neuronal activity; receptors for many neuropeptides are present on the postsynaptosome membrane [98]. On the other hand, neuropeptides are present together with traditional neurotransmitters in the same nerve endings [61], which contradicts the classical rule formulated by H. H. Dale [40], which became dogmatic: one neuron — one neurotransmitter.

Modern scientists do not deny the wide spectrum of functional activities of neuropeptides as bioregulators [3,4,85], but are liable to think that these compounds can also act as neurotransmitters in the course of synaptic transmission, *i. e.* stimulate or inhibit nerve transmission realized through traditional neurotransmitters [61,98]. Pathogenesis of some CNS diseases, *e. g.* paranoid schizophrenia can be explained from this viewpoint [99].

The syndrome characteristic of this nosological entity (hallucinations, delirium, disorders in higher nervous activity) is easily induced by amphetamine (phenamine), causing excessive release of dopamine from presynaptic terminals of the neuronal dopamine system, specifically, from the mesolimbic system closely connected with higher nervous activity [38,39]. On the other hand, this syndrome can be removed by neuroleptics used in psychiatry [33]. It was found that along with traditional neurotransmitter dopamine, presynaptic terminals of the dopamine system neurons coexpress vasoactive intestinal peptide inhibiting dopamine release from nerve terminals [61]. It is therefore suggested that the neuropeptide or its metabolites (as neuropeptides are short-living compounds) normally regulate dopamine release from nerve endings. If the modulating function of the neuropeptide is impaired, dopamine is released in excess, which causes schizophrenia syndrome. These data elucidate the pathogenesis of paranoid schizophrenia and determine the treatment strategy.

Hence, neuroendocrinology, born at the interface of different disciplines, became an important branch of neurosciences. It disclosed the endocrine functions of the brain and shed light on some of its mysteries [1].

Era of Neuroimmunoendocrinology

When discussing the unity of the nervous and endocrine systems we mentioned that neurons, retaining specific organization and function (generation and propagation of nerve pulses) can simultaneously function as endocrine cells, but the same is true for immune cells. Participating in homeostasis regulation through specific immune mechanisms, these cells express receptors to many signal molecules mediating the effects of the neuroendocrine system [28-30,66,75,83,106] and synthesize some evolutionally ancient (conservative) peptides [49,55-57]. Among them are neuropeptides, tachykinins, insulin, propiomelanocortin (ACTH precursor), growth hormone, and prolactin, whose receptors belong to the hemopoietin family, including also receptors to interleukins, erythropoietin, granulocyte-macrophage colony-stimulating factor [71]. Activities of the immune defense and growth hormone are known to decrease during aging. Similarly the secretion of prolactin and oxytocin decreases and that of sex steroids (estrogens and progesterone) increases in physiologic states involving depression of the immune system, *e. g.* during pregnancy. After delivery, when the secretion of steroid hormones decreases and the

immune system is activated again, the secretion of prolactin and oxytocin increases. Suppression of the immune defense function with aging is paralleled by increased secretion of ACTH.

In this connection, neuropeptide molecules and molecules of their protein carriers (neurophysins), synthesized in thymic epithelial cells, attract special attention. V. Greenen *et al.* [57], summing up studies thereof, hypothesized a possible role of neurophysin as a molecule coexpressed in the thymus with HLA-I antigen and contributing to "training" of T lymphocytes developing in the thymus [34].

Analysis of similarity in the structure of the nervous and immune systems shows that both systems consist of numerous phenotypically different cells arranged in complex networks [32,68,69,84]. Within such a network the cells are related and function by the feedback mechanism, when an adequate stimulator serves as the triggering signal and the final response is aimed at providing a useful result. The difference is that nervous system cells are rigidly fixed in space, while immune system cells are constantly moving and interact with each other only for a short time. Functional similarity of the nervous and immune cell can be traced in some diseases.

The phenomenon of glutamate death of neurons is well known. It is observed in brain neurons stimulated through glutamic acid or glutamate, the predominant neurotransmitter of the brain. This phenomenon is observed in ischemic parts of the brain, for example during stroke caused by thrombosis or embolism. High-frequency discharges create a glutamate cascade in glutamatergic neurons, which does not permit potential-dependent calcium channels to close and leads to excessive Ca^{2+} entry into postsynaptic neurons. Binding there to its receptor calmodulin, calcium activates nitroxide synthase. In the presence of NADPH, activated enzyme catalyzes transformation of L-arginine into gaseous intermediate metabolite nitric oxide (NO), which is diffusely released from the cell and reacts with the neighboring neurons. The receptor target of NO is iron atom in the active center of guanylate cyclase molecule. Binding to iron, NO initiates conformation changes in the enzyme molecule, which leads to its activation (allergostatic effect) and finally to the production of cGMP functioning as the second messenger. The latter, interacting with G proteins, stimulates the neighboring neurons. All this allows us to regard NO as a nontraditional neurotransmitter [31,42]. Unlike other neurotransmitters it is not stored in the synaptic vesicles, but released diffusely, and not at the site of synaptic contacts (so-called volume transfer).

Hence, glutamate death of neurons is similar to posttetanic potentiation, which facilitates synaptic transfer. In both cases discharges following each other do not allow the potential-dependent calcium channels to close, which leads to massive calcium entry into the cells and, hence, to increased release of neurotransmitter, in our case glutamate. Glutamate bombing of arginine-containing postsynaptic neurons in ischemic brain regions results in increased NO production, whose excessive release has a toxic effect on the adjacent neurons. In this case NO-synthesizing neurons are similar to activated macrophages. The following experiments illustrate this assumption.

Experimental stroke was simulated in chick embryo cerebrocortical neuron culture. Up to 90% neurons died after 5-min exposure to NMDA (N-methyl-D-aspartate, highly affine to the glutamate receptor subtype). Inhibitors of NO synthesis (methyl- or nitroarginine or hemoglobin) effectively protected neurons from the glutamate cascade, neuronal death being thus decreased by 73% [35,44,45].

Hence, after stimulation with moderate doses of glutamate, NO stimulated the production of cGMP in the adjacent neurons and acted as a neurotransmitter. After stimulation with high doses of glutamate the neurons producing NO act as macrophages. Surprisingly, that NO-producing neurons are protected from the neurotoxic effect of NO. Different mechanisms of protection of these neurons are hypothesized. According to one hypothesis, SOD which removes superoxide anions interacting with NO to form a highly toxic compound peroxynitrite performs the protective function [43]. Another hypothesis is based on the fact that oxidized free radical (NO^+) is characterized by a neuroprotective effect [77]. Presumably, macrophages are protected from free-radical compound NO which they produce through the same mechanisms.

A demonstrative example of interactions between the neuroendocrine and immune systems is stress reaction. This biological defense reaction develops in response to a wide spectrum of aggressive environmental factors, such as bacterial, thermal, painful agents, immobilization, gravitation, psychoemotional exposures, *etc.* The neuroendocrine hypothalamus-pituitary-adrenal cortex system is activated by all these factors: secretion of corticoliberin (corticotropin releasing hormone, CRH) in the hypothalamic paraventricular nucleus (PVN) increases, which results in activation of ACTH secretion in the pituitary anterior lobe, which leads to increased glucocorticoid secretion in the adrenal cortex. H. Selye [96], who was the first to describe this reaction, noted that the immune system was not indifferent to stress. Mechanisms by which the immune system is involved in stress reactions were investigated later [6,7,41].

Interleukin-1 (IL-1) is released from macrophages in response to pathogens. This immune peptide penetrates into the brain via the blood-brain barrier through

special "windows" for such substances. Among such sites are the system of circumventricular organs [74] possessing specific mechanisms of cytokine transport [22,23,48,51]. In the brain IL-1 stimulate CRH secretion in neuronal population of hypothalamic PVN [70, 101], this process depending on the presence of prostaglandin (PG) E_2 [26,78]. CRH, in turn, stimulates the secretion of ACTH in the pituitary, which leads to increased secretion of glucocorticoid hormones in the adrenal cortex. During increased secretion the adrenals inhibit IL-1 secretion in macrophages thus suppressing the immune response if it is excessive. Thus, we see here pure negative feedback mechanisms, in which the immune peptide functions as the trigger and neuropeptide and endocrine hormones as the performers [25,27].

We observed these mechanisms for the first time when investigating the hypothalamic regulation of the pancreatic insulin-secreting function.

It was observed long ago that the regulation of one of the peripheral endocrine functions, insulin secretion by the pancreatic gland, cannot be explained within the framework of common notions on the neurohumoral regulation along the hypothalamus—pituitary—target organ axis. Insulinotropic hormone was absent in the pituitary component of this axis. On the other hand, stimulation of the vagus nerve or injection of acetylcholine or its antagonists led to insulin release into the blood, while crossing of the vagus nerve or injection of acetylcholine antagonists had an opposite effect [2]. This and rich cholinergic innervation of the pancreatic islets suggest that the hypothalamic regulation of pancreatic function (hypothalamic regulation of this function caused no doubts) can be realized without involving the pituitary through the nervous conduction route connecting the hypothalamic neurons with pancreatic islets by the vagus system. In order to prove this, it was necessary to find the descending hypothalamic axons, to elucidate from which nucleus they descend, and to find out whether they form synaptic bonds with the stem center of the vagus nerve.

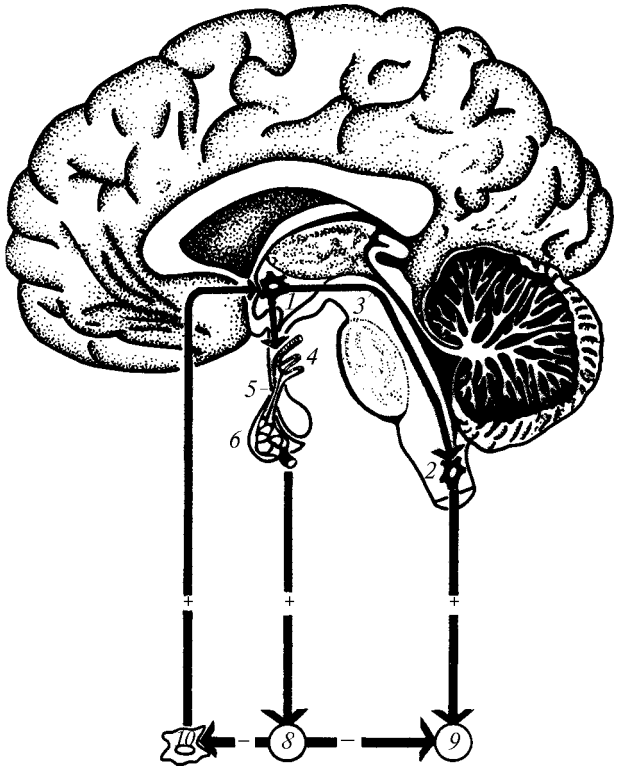

Fig. 1. Feedback mechanisms underlying the interactions between the neuroendocrine and immune systems during hypothalamic regulation of carbohydrate homeostasis. *1*) hypothalamic paraventricular nucleus; *2*) dorsal vagus nucleus in medulla oblongata; *3*) paraventriculovagus system of nerve bonds; *4*) capillary roots of portal veins in the pituitary stem; *5*) pituitary portal veins; *6*) cleavage of portal veins in the anterior lobe of the pituitary into secondary capillary network; *7*) posterior lobe of pituitary; *8*) adrenocortical cells secreting glucocorticoids; *9*) insulin-secreting β-cells of pancreatic islets; *10*) macrophages secreting interleukin-1.

Many-year studies proved experimentally the existence of the descending hypothalamo-vagus pathway. It descends from neurons of the hypothalamic PVN, synaptically transfers to neurons of the vagus dorsal nucleus in the medulla oblongata, and reaches the pancreatic islets via the vagus nerve [13,14]. We called this route **paraventriculovagal** (Fig. 1). By the time we came to this conclusion, similar data were obtained by other scientists by tracer methods (injection of isotope label to the site of presumable descending axons or horse radish peroxidase to the site of their presumable ending) [13].

The results of subsequent studies were rather intriguing. Stimulatory effects come to pancreatic β-cells via the paraventriculovagus pathway (Fig. 1). At the same time CRH is secreted in one of PVN neuronal populations involved in the formation of the paraventriculovagus pathway; CRH reaches the pituitary via the portal vessels and induces the secretion of ACTH and glucocorticoids. These hormones enter the pancreatic islets by the humoral pathway and inhibit insulin release. Such double regulation is common in the regulation of endocrine functions. However the feedback mechanisms involved in this regulation are not confined to the neuroendocrine system, but affect the immune system components as well. Macrophages stimulated by low glucocorticoid doses release IL-1, which, as was previously mentioned, penetrates through the hematoencephalic barrier and stimulates CRH secretion in PVN. Systemic injection of IL-1 had a similar effect, the increase in CRH secretion depending on PGE_2 [46,50,86,105]. This latter fact suggests an alternative mechanism of IL-1 effect on PVN neurons secreting CRH: IL-1 in cerebrovascular endothelial cells stimulates the synthesis of PG (including PGE_2) through cyclogenase, and PG mediate the stimulating effect of IL-1 on CRH secretion.

In view of all this, we should like to review the facts indicating the capacity of hypothalamic neurosecretory cells to express not only IL-1 receptors [20, 21,52], but IL-1 as well, which was shown for rats and humans [19]. Moreover, astrocyte glial cells secrete interferon, which increases IL-2 expression in cerebral nervous structures [104].

Further studies of the mechanisms of interactions between the neuroendocrine and immune systems were carried out in cooperation with Department of Endocrine System Physiology headed by Dr. G. Aguilera at one of National Health Institutes in Bethesda, USA. We studied the effect of immune stress on the hypothalamo—pituitary—adrenal system of rats under conditions of acute and chronic stress. Immune stress was induced injection of endotoxin antigen (LPS). Acute stress was induced by a single intraperitoneal injection of LPS (250 μg/100 g) and chronic stress by injections of the antigen in ascending doses (25-250 μg for 13 days). The activity of the neuroendocrine axis was evaluated by the time course of expression of appropriate mRNA (CRH mRNA in PVN, proopiomelanocortine (ACTH precursor) mRNA in the pituitary, and 11β-hydroxylase (key enzyme in corticosterone biosynthesis) mRNA in the adrenal cortex) by *in situ* hybridization and by the level of circulating hormones (ACTH and glucocorticoids), which were measured in the plasma by routine methods.

Activation of all components of the neuroendocrine axis, *i. e*. increased secretion of CRH in PVN neurons, ACTH in the pituitary, and glucocorticoids in the adrenal cortex, were observed in animals during acute stress. A paradoxical effect was observed during chronic stress: CRH secretion in PVN was sharply suppressed, while the secretion of ACTH in the pituitary and of glucocorticoids in the adrenal cortex remained at the same level as in acute stress. A similar picture is observed in some chronic inflammatory autoimmune diseases, such as arthritis induced by adjuvant injection, systemic lupus erythematosus, and allergic encephalomyelitis [63,65,87,97]. Suppressed synthesis of CRH in such cases can be due to long inhibitory effects of increased content of glucocorticoids or to imbalance of neurotransmitters in the hypothalamus [36, 62,64]. In any case, suppression of synthesis of the central neurohormone of the hypothalamo-pituitary-adrenocortical axis is associated with paradoxical activation of its pituitary-adrenal component.

The situation partly reminds that observed in the middle of the last century during investigation of the pathogenesis of diffuse toxic goiter (Graves' disease). This disease is characterized by hypersecretion of thyroid hormones in the presence of normal level of the pituitary thyrotropic hormone (TTH). This paradoxical fact was hypothesized to be due to a long-acting and stimulant binding to thyrocyte receptors competitively replacing TTH [11]. Such a stimulant was identified. It was IgG whose soluble form circulated in the blood as thyrostimulating antibodies [73]. Their appearance in the blood was attributed to disorders in immune system and was regarded as a manifestation of autoimmune pathology.

Such a precedent admits that cytokines, *e. g*. IL-6 secreted by follicular astrocytes of the pituitary anterior lobe, can act as ACTH stimulators in chronic immune stress [16,18,72,88,100]. The secretion of cytokines in the anterior lobe of the pituitary and in PVN neurons is PGE_2-dependent and stimulated by LPS (like in macrophages) [10,18,19]. Cytokines secreted in the pituitary can cause proliferation of various tropic (including ACTH-secreting) cells and induce the respective pituitary adenomas, *e. g.* adrenocorticotropinoma [15,82]. The latter fact can shed light on the pathogenesis of Icenko—Cushing disease. A characteristic feature of this etiologically complex disease is the presence of pituitary adenoma (adrenocorticotropinoma), high levels of ACTH and glucocorticoid secretion, and no clear-cut evidence of increased CRH secretion.

A chronic autoimmune disease — arthritis induced by an adjuvant (heat-killed *Mycobacterium butyricum* culture) can serve as a model of autoimmune disease demonstrating the involvement and interactions of three regulatory systems in the mechanisms of disease development. This disease is associated with a paradoxical suppression of CRH synthesis, paralleled by increased production of ACTH and glucocorticoids. Response to psychoemotional stress is decreased under such conditions [12]. However study of the reaction of the hypothalamo—pituitary—adrenal axis to immune stress (single intraperitoneal injection of LPS in a dose of 200 μg) in animals with arthritis [5,58] showed pronounced activation of all its components, including the hypothalamic component (Fig. 2) (the study was performed in cooperation with Dr. G. Aguilera from National Institutes of Health, USA, Drs. S. Lightman and M. Harbuz from University of Bristol, UK, and Dr. F. Tilders from Free University of Amsterdam, Netherlands).

This uncommon reaction attracted out attention, and we investigated the synthesis and release of the most significant cytokines IL-1 and IL-6 into the blood. Injection of LPS to animals with arthritis sharply increased cytokine level in the blood and expression of cytokine mRNA in the brain and peripheral nerves. Such universal reaction of the immune and neuroendocrine system was termed as hypersensitivity of the hypothalamo-pituitary-adrenal axis to immune stress in chronic autoimmune disease [5,58] and can be clinically used for evaluating the disease severity (Fig. 3).

One more example of neuroimmunoendocrine relationships is development of diabetes mellitus. Disorders of the carbohydrate homeostasis are often observed in middle-aged and elderly people after psychoemotional stress (death of a close relative, conflicts in the family or at work, *etc.*). Increased blood sugar level is one of the early signs. This signal can be a transitory or stable symptom, which later transforms into characteristic syndrome (impaired glucose tolerance, hyperglycemia, glycosuria, high content of glycated hemoglobin, presence of specific antibodies in the blood, *etc.*). A possible pathogenetic mechanism is as follows: stress reactions are associated with appearance of thermal shock bacterial proteins, which are foreign for the organism, as they are not presented by T lymphocytes during the development of acquired immunity. When they bind to HLA-II antigens on the surface of macrophages and insulin-secreting β-cells, they are recognized by T lymphocyte receptors and initiate an autoimmune response aimed at destruction of β-cells of pancreatic islets. I. R. Cohen [37] and his colleagues from Veismann Institute (Israel) demonstrated it by immunization of normal mice with thermal shock proteins and transplanting T lymphocytes from immunized mice to healthy animals.

The theory of molecular mimicry, most persuasively explaining the mechanisms of autoimmune disease, received recently much attention [17,37]. According to this theory, molecules of a pathogenic agent entering the body can show chemical and conformation similarity to host molecules inserted in endocrine cell membranes. In this case, T helpers recognize them as a foreign antigen and initiate autoimmune aggression against one's own cells using the entire armory of attack means. The scheme of events is as follows. A characteristic feature of pancreatic islet β-cells is the presence of HLA-I and HLA-II antigens on the surface, and therefore they become targets for both helper and cytotoxic T lymphocytes. When T helper receptors recognize a foreign antigen associated with HLA-II on the presenting cell, T lymphocyte activation takes place, consisting of several processes. Tyrosine kinases are activated, stimulating the activity of phospholipase C. The latter enzyme catalyzes hydrolysis of membrane phospholipids (phosphorylated precursors of second messengers, specifically phosphatidylinositol diphosphate) with formation of diacylglycerol and inositol-3-phosphate. The latter promotes release of Ca ions from cell depots (from mitochondria and smooth endoplasmic reticulum), which interact with calmodulin and activate protein kinase C (or C-kinase), a process in which diacylglycerol is involved as well. C-kinase activates the transcription factor of the gene coding for synthesis of IL-2, an important immune system mediator. This cytokine functions as growth factor

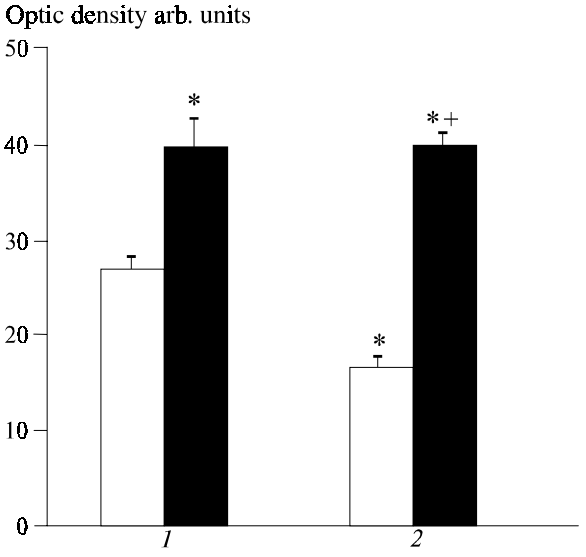

Fig. 2. Expression of corticotropin-releasing hormone mRNA in the small-cell part of hypothalamic paraventricular nucleus after injection of LPS in rats with chronic autoimmune arthritis induced by injection of adjuvant. *1*) control; *2*) autoimmune arthritis; light bars: initial values; dark bars: after injection of LPS. $p<0.01$: **vs.* initial values in the control; $^{+}$*vs.* initial values in animals with autoimmune arthritis.

in the immune system; it induces the proliferation of T helpers, as a result of which the autoreactive immune response unfolds on a wide scale. In addition, IL-2 effect leads to proliferation of another T lymphocyte pool, cytotoxic cells, recognizing HLA-I associated foreign antigen on the surface of β-cells. As a result of activation, cytotoxic T lymphocytes release perforine, which creates porous structure in β-cell membrane. Ca and Na ions enter the cells through these pores. Each mole of sodium binds 5-6 moles water, which leads to hydropia of β-cell cytoplasm and swelling of mitochondria. Mitochondrial damage impairs cell energy and energy-dependent pumps, pumping excessive Ca^{2+} and Na^{+} from the cells. Ca^{2+} excess exerts a toxic effect and Na^{+} excess impairs cell osmolarity. As a result, part of β-cells die by hydropic transformation and the other part by apoptosis. In this case other enzymes released by activated cytotoxic T lymphocytes (granzymes) enter the cell through porous membrane. Granzymes interacting with proteases similar to IL-1 converting enzyme activate endonucleases, which cut nuclear chromatin into individual nucleosomes detected in apoptotic corpuscles [47]. However the armory of means of attack is not exhausted by this: IL-2 enhances the proliferation of B lymphocytes and stimulates antibody production in them. Entering the blood, antibodies opsonize macrophages by stimulating release of highly reactive free-radical compounds (NO) and IL-1. These factors augment destruction of β-cells. Finally, antibodies activate the complement system circulating in the blood (potent

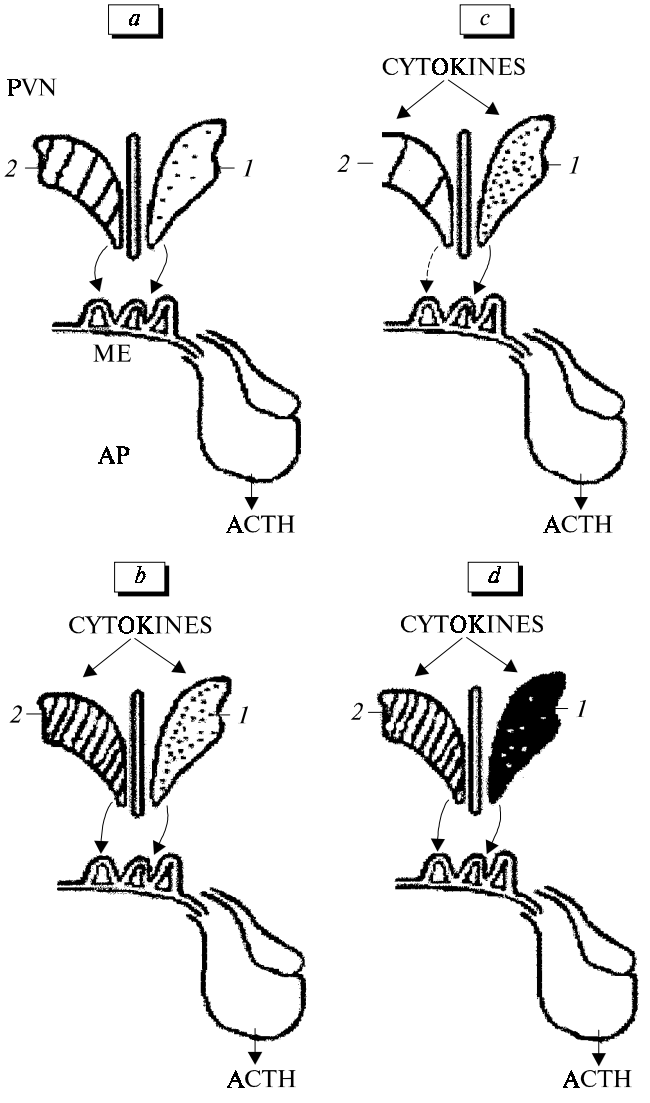

Fig. 3. Correlations between activity of neuroendocrine system and cytokine production in health (*a*), acute inflammation induced by LPS (*b*), chronic inflammation - arthritis (*c*), and combined chronic and acute inflammation (*d*). *1*) vasopressin; *2*) corticotropin releasing hormone (corticopliberin); ACTH: adrenocorticotropic hormone; AP: anteriopr pituitary; PVN: small-cell part of paraventricular nucleus; ME: median eminence of the neuropituitary. Density of cross-hatching of areas *1* and *2* reflects the level of respective hormone.

system of proteolytic enzymes with cascade mechanism of action), augmenting β-cells destruction.

Thus, massive death of insulin-secreting β-cells occurs. When 80-90% cells die, the remaining pool of secretory cells can no longer maintain vitally important homeostatic function, and injections of insulin are needed for its compensation. Insulin-dependent diabetes mellitus develops.

Autoimmune nature of endocrine disease is observed not only in patients with Graves' disease and diabetes mellitus, but in patients with primary chronic adrenocortical insufficiency (Addison's disease) and gonadal hormonal dysfunction leading to sterility as well.

Nervous cells often become the object of aggression. In diabetes insipidus autoimmune aggression is directed to the hypothalamic neurons secreting vasopressin characterized by antidiuretic activity [95]. In disseminated sclerosis the foreign antigen is the major myelin protein coating nerve fibers as an interface membrane [37]. Myelin acts as an insulator preventing release of ionic currents. When this insulator is damaged, nerve pulses fade and cannot reach their target (muscle cells). As a result, patients die from respiratory muscle paralysis. The same grave outcome is observed in severe myasthenia (*myasthenia gravis*), when protein receptors to neurotransmitter acetylcholine, released in the area of the neuromuscular synapses, serve as the foreign antigens [76].

Conclusion

The understanding of deep mutual relationships between the nervous, endocrine, and immune systems is difficult if we regard the mechanisms of their interactions at the level of highly specialized systems in mammals. The history of research of these systems is full of dramatic collisions which puzzled the scientists. Researchers often faced facts that could not be explained from the viewpoint of common logical thinking: despite their highly specialized functional organization, nerve cells behaved like endocrine ones, while endocrine cells (APUD cells) displayed capture biogenic amine precursors, decarboxylate them, and release as traditional neurotransmitters catecholamine or indole amines. We have to acknowledge that the immune system cells possess signs of organization and function typical of nervous and endocrine system cells.

It is therefore necessary to recollect here the sources of development of regulatory systems with complex organization, whose functions were initially simplified in one-celled organisms. One-celled organisms had to face aggressive environmental factors and develop adaptation mechanisms for defense and adaptation. Using simple analyzers, they developed avoidance reaction, making use of the contractile activity mechanism intrinsic for one-celled organisms. Phagocytosis and production of substances destroying and digesting pathogens became other defense means. The capacity to secrete bioactive substances, hormones maintaining homeostasis, belongs to important evolutional acquisitions. All these events took place in one and the same cell, and cooperations of these functions developed, which imprinted (let us call it **biological imprinting**) at later stages of evolution, when one-celled organisms became many-celled, and the above-listed functions, retaining the cooperation principle, were reproduced in highly specialized functional systems. This explains why two other functions are involved in the realization

of response of each of the regulatory systems; similarity in their organization facilitates the cooperation. The mechanisms of immune response are an important component of the neuroendocrine activity, and the immune response requires coordinated involvement of the nervous and endocrine systems. From this viewpoint it is easy to understand the origin of autoimmune abnormalities in diseases involving the nervous and endocrine systems.

Hence, new science neuroimmunoendocrinology is a perspective trend in research of physiological basis of vital activity and pathogenetic mechanisms of diseases involving the nervous and endocrine systems.

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