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The peptide molecular links between the central nervous and the immune systems

Review Article

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Summary. The central nervous system (CNS) and the immune system were for many years considered as two autonomous systems. Now, the reciprocal connections between them are generally recognized and very well documented. The links are realized mainly by various immuno- and neuropeptides. In the review the influence of the following immunopeptides on CNS is presented: tuftsin, thymulin, thymopoietin and thymopentin, thymosins, and thymic humoral factor. On the other side, the activity in the immune system of such neuropeptides as substance P, neurotensin, some neurokinins, enkephalins, and endorphins is discussed.

Keywords: CNS–immune system molecular links – Immunopeptides – Neuropeptides – Thymic peptides

Introduction

For many years the central nervous and the immune systems were considered as two completely autonomous, working independently systems of the animal body. However, the increasing amount of observation suggested that various mental disorders and depressions significantly influence some malignant diseases in humans. Some illustration of such a situation can be found in a short review of Leonard (1988), e.g. the observation that HIV infection can result in dementia of patients who recover from various infections suggests that there is a direct biological link between the brain and the immune system.

As early as in 1936 Selye observed that stress induces the atrophy of thymus (Selye, 1936). In 1964 Solomon and Moos introduced the term psychoimmunology for the science which interprets stress, emotions and immunological dysfunctions (Solomon and Moos, 1964).

In this connection we would like to remind about an old observation of three anatomopathologists of the Polish University in Wilno (now Vilnius, Lithuania) done in the thirties of the XIX century. Thus, Professor A. Bielkiewicz has observed a hypertrophy of thymi in the organisms of suicides and mentally defected people. The observation was confirmed by Siewruk and Leonow (see Belke and Kramer, 1853). In 1842 Leonow presented a report entitled *Commentatio a se elaboratum de glandulo Thymo, respectu physiologica et medico-legali*, in which these observations were summarized (Bieliński, 1886). This observation is in accord with another old discovery of Cantacuzene (1898). The author observed a suppressive effect of morphine on the phagocytosis, which of course evidences (in the light of contemporary knowledge) the presence of brain enkephalin receptors on the immune cells. These observations could be quoted in the context of the new results concerning the suppressive effects of morphine on the mitogen induced proliferation of spleen and blood lymphocytes (Lysle et al., 1993).

In the recent years many review articles were published describing various aspects of immune-neuroendocrine interactions. Among them those of Owens et al. (1994), Saviano and Dardenne (1995), Herman (1998), Mentlein and Kendall (2000), Saviano and Dardenne (2000), and Masek et al. (2003) could be quoted here. Several articles connected to this and related subjects were published also in Amino Acids journal (e.g. Abiko and Sekino, 1993; Mihelic and Voelter, 1994; Winkler et al., 2002; Janin,

2003). For this rapidly developing new branch of biosciences the name neuroendocrineimmunology (NEI), or immunopsychopharmacology is now used.

It was established in 1984 by Blalock and Smith (1984) that the immune system and brain speak a common biological language, i.e. that cells of the immune system are able to produce neuropeptides, whereas the neurons and glial cells could release a variety of cytokines. In the agreement with this finding the receptors for both these types of molecules appear in cells of the immune, as well as the neuroendocrine system. Numerous studies concerning this problem were reviewed in 1994 by Blalock (1994).

Neuroimmune interactions are bidirectional. Many different compounds: cytokine proteins, polypeptide hormones, neuropeptides and neurotransmitters may participate in the signal transmission between the immune and the central nervous system.

In this paper we have given the attention to the peptides which are important for neuroimmune communication. Therefore, the picture we try to draw here is not complete, and as such it is oriented rather to meet the expectations of a peptide chemist.

1 Immunopeptides

1.1 Tuftsin

According to our knowledge, tuftsin was the first immune peptide for which the distinct influence on the functions of CNS was found. Tuftsin is a tetrapeptide with the sequence Thr-Lys-Pro-Arg. It was isolated in 1970 by Najjar and Nishioka from leucophilic fraction of IgG protein (Najjar and Nishioka, 1970). The amino acid sequence of the peptide was established by the same research group (Nishioka et al., 1972). Tuftsin is a 289–292 sequence of the CH2 domain of the Fc fragment of leucokinin molecule. According to the results of Najjar's group, it is cleaved from the protein carrier by the successive action of two enzymes: splenic tuftsin endocarboxypeptidase, and phagocyte enzyme – leucokinase (Najjar, 1980).

The principal biological activity of tuftsin consists in activation of phagocytosis realized by granulocytes and macrophages. It also activates pinocytosis, increases respiratory burst of phagocytic cells thus stimulating their bactericidal activity, and destroys the neoplasmatic cells. The works on biological activity of tuftsin and its analogs were reviewed in 1999 by Siemion and Kluczyk (1999).

In 1980 Prof. Herman in collaboration with our research group found that tuftsin administered intracerebroventricularly (i.c.v.) to rats elicits analgesia of 20 min duration (Herman et al., 1980, 1981). We have concluded there that tuftsin can constitute ''a link between immunobiological responses and the central nervous system, since there is the possibility that tuftsin continuously synthesized for stimulation of phagocytes affects the central nervous system''. Such a conclusion was met at that time with some disbelief of the Editor of ''Experientia''.

It was found later that the effect of tuftsin is not altered by naloxone, and that its D-Arg^4-analogue appears to be a very potent analgesic agent. Among the partial sequences of tuftsin, C-terminal dipeptide Pro-Arg revealed the evident analgesic action in both hot-plate and tail-flick immersion tests (Herman et al., 1985). Further studies confirmed a role of the Pro-Arg sequence in generating analgesic activity of tuftsin (Nawrocka et al., 1988). Tuftsin does not interact with opiate receptors. However, bradykinin, injected i.c.v. to rats and mice decreased the antinociceptic action of tuftsin (Herman et al., 1983).

Some tuftsin-like fragments of viral proteins (hepatitis virus S-protein, human adenovirus type 2 protein, human immunodeficiency virus HIV-1 and HIV-2 proteins) were also tested in respect to their analgesic activities (Obuchowicz et al., 1993).

It was also stated (Herman, 1983) that tuftsin affects the exploratory and locomotor activity of rats in a biphasic manner. After a short period of depression, the stimulation of these activities was observed.

Hypothalamic peptide, neurotensin, which antagonizes the antinociceptive effect of enkephalines, is an agonist of tuftsin in induction of analgesia, which suggests that the peptide modulates the function of enkephalinergic neurons and the central effects of tuftsin in an opposite way (Herman et al., 1984). It seems worth noting that in the neurotensin sequence Glp-Leu-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu (Glp – pyroglutamic acid) there is a tuftsin-like fragment Asn-Lys-Pro-Arg and this could be the source of agonistic properties of neurotensin in respect to the tuftsin action.

The central effects of tuftsin were observed also by other research groups. Thus, Aronowski et al. (1985) showed that tuftsin and [Lys4]-tuftsinyl-tuftsin attenuate the withdrawal behavior in morphine-dependent rats. The antinociceptive effect of [Hyp3]-tuftsin was studied by Galasik-Bartoszek et al. (1991). Nicolaides et al. examined the effect of i.c.v. administration of tuftsin on the acetic acid-induced writhing in mice (Nicolaides et al., 1985). Valdman et al. observed the enhanced locomotor

activity and aggressiveness in mice after i.p. injection of large doses of tuftsin, as well as the decreased immobility of rats in the despair test and the increase of their exploratory activity (Valdman et al., 1981, 1982). The influence of tuftsin on the locomotor activity of animals was also investigated by Levretskaia et al. (1981). Semenova et al. (1988a, b) found that i.p. injection of tuftsin to rats induces the increase in memory traces stability during a 30 day period and affects the learning and exploratory behavior. Kamenskii et al. (1989) showed that tuftsin selectively affects the central nervous system when administered intranasally. The effect of tuftsin on the bioelectrical activity of brain structures was studied by Veskov et al. (1995) and Popova et al. (1996). In particular, these authors showed that tuftsin augments catecholaminergic and suppresses serotonin activity in the sensorimotor cortex and caudate nucleus. The effects of tuftsin on the brain monoaminergic system and animal behavior was studied by Seredenin et al. (1995). It was found that the injections of tuftsin analog TP-7 (heptapeptide Thr-Lys-Pro-Arg-Pro-Gly-Pro) normalize the serotonin level in the brain of rats with chronic deprivation of serotoninergic system. The same group investigated the influence of tuftsin on learning, exploratory activity, emotional behavior, and hypothalamic monoamine content in Wistar rats with different resistance to stress. In rats with a high resistance to stress the decrease of hypothalamic noradrenaline level and the increase of dopamine and serotonin level was observed. The opposite effects were stated for low-resistant animals (Ismailova et al., 1998). The positive emotional effects and antistress actions of tuftsin and some of its analogs given i.p. were tested recently on rats and mice by Kozlovskaya et al. (2003). Morphochemical changes in the visual and sensorimotor cortical neurons of rats exposed to tuftsin were studied by Chebotareva (1990).

The influence of tuftsin on the metabolism of various brain structures was studied by Begum (1985), Begum and Maksimovich (1985), Ilin et al. (1986), and Kamysheva (1989). It was also found that cytosolic aminopeptidase in the monkey brain cleaves amino acid residues sequentially from the N-terminus of tuftsin (Ramamoorthy and Balasubramanian, 1992).

The ^{99m}Tc-RP128 radiopharmaceutical (constructed of Tc-cation and tripeptide Gly-Ser-Cys(Acm) linked by a Gly residue to pentapeptide Thr-Lys-Pro-Pro-Arg which binds *in vivo* to the tuftsin receptor, was used recently for the detection and quantitation of inflammation in the central nervous system (Paul et al., 2000).

The interesting results were also obtained for tuftsin N-terminal tripeptide Thr-Lys-Pro. For this tripeptide an inhibitory activity in respect to macrophage migration and generation of O_2^- by stimulated macrophages was detected by Auriault et al. (1983). Whiteley et al. (1998) found that intravitreal injections of this peptide increase the recovery of pupillary light reflex, following retinal ganglion cell axon regeneration through peripheral nerve grafts in adult rats. This result was confirmed by Raibon et al. (2002) who investigated also the microglial changes accompanying this process.

Recently Wang et al. (2003) found that the 1–3 fragment of tuftsin which acts as a macrophage/microglial inhibitory factor, plays a protective role in the animal model of intracerebral hemorrhage.

1.2 Thymic peptides

The role of the central lymphoid organ of the immune system is played by thymus. The thymus-dependent lymphocytes (T-cells), maturated in the thymus, are supplied to the peripheral lymphoid tissues, spleen, and the lymph nodes.

The cellular and molecular interactions of thymus with endocrine organs and the nervous system were reviewed recently by Kinoshita and Hato (2001).

Thymus is innervated by autonomic nerve fibres and this nerve system is related to the control of pre-T-cell migration from the bone marrow into the thymic parenchyma and the regulation of thymocyte differentiation and maturation. The role of the central regulator for the autonomic nerve system which innervates the parenchyma is played by hypothalamus. It is speculated that the surgical deprivation of thymus causes the disorder of hypothalamic functions, evoking the cognitive deterioration of CNS. In agreement with this theory the deterioration of learning and memory ability was observed in thymectomized mice (Zhang, 1994).

Among more than 30 peptides produced in thymus, known as ''thymic hormones'', some as thymulin, thymopoietin, and thymosin α and β are known for their ability to influence the central nervous system.

1.2.1 Thymulin

Thymulin is a nonapeptide with the sequence: Glp-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH. It was obtained by Bach et al. (1975) and denoted as ''facteur thymique serique'' (FTS). The peptide is active in the form of zinc complex. For this active form of the compound Bach proposed afterwards the term ''thymulin'' (Dardenne et al., 1981).

Thymulin is produced within the reticulo-epithelial cell network of thymus, and secreted to the blood, among other thymic regulatory peptides. The majority of these peptides is, in fact, not thymus specific (Hadden, 1992). Only the zinc-thymulin complex is qualified as a regular secretory product of thymic epithelial cells (Hadden, 1993). The thymic serum levels were determined by RIA assay to be about 50 pg/ml, however, the data from amino acid analyses suggest a content of 100 pg/ml. In the opinion of Bach et al. (1980), these data are underestimated and it is likely that the real serum levels of thymulin range between 0.5 and 5 ng/ml.

The concentration of thymulin in rat and human serum shows a 24 h rhythm with the increase at night (Molinero et al., 2000). Recently, the circadian and circannual fluctuation of thymulin level was investigated by Labunets (2001) in respect to the age of animals.

The principal biological role of thymulin consists in immunomodulation. It induces various T and NK cell activities and promotes the differentiation of T cells precursors (Bordigoni et al., 1984; Incefy et al., 1980).

The zinc binding induces the conformational changes within the thymulin molecule that are necessary for the expression of biological activity; the NMR studies showed also that this form of the peptide possesses a unique spatial structure (Dardenne et al., 1985). There is, however, an increasing amount of data which argument that thymulin is a part of integrated homeostatic network linking the immune system to the nervous and endocrine systems. Nowadays the existence of neuroendocrine – thymic axis is well documented. A short review concerning this subject was very recently published by Goya et al. (2004). The thymic epithelial cells possess a specific growth hormone, as well as prolactin receptors (both produced in pituitary) (Besedovsky et al., 1974; Dardenne et al., 1991). In agreement with this finding the human growth hormone can stimulate thymulin release from thymic epithelial cell lines *in vitro* (Timsit et al., 1992). Treatment of old mice with ovine growth hormone restores also the thymulin serum level (Goya et al., 1993). Prolactin can also stimulate the thymulin secretion *in vitro* as well as *in vivo* (Dardenne et al., 1989). Folch et al. (1986) showed that the hypothalamus extract induces the reappearance of thymulin in old mice. Afterwards similar experiments were done by Goya et al. (1995). These authors reported that hypothalamic and pituitary extracts from young mice stimulate the thymulin production, whereas the extracts obtained from old mice demonstrate the decline of the stimulatory activity.

As it was shown recently by Giacconi et al. (2003), the level of active thymulin can be also regulated by metallothionein-III isoform, a zinc binding protein produced in hippocampus. The protein may sequester and release zinc, influencing the zinc ions bioavailability which can be tested by determination of total thymulin/active thymulin in the plasma.

It seems worth noting that the reduction of thymulin activity was observed in the patients with Crohn's disease and acute lymphoblastic leukemia, in which zinc deficiency is noticed (Brignola et al., 1993; Mocchegiani et al., 1994).

On the other hand, thymulin might modulate the secretion of adrenocorticotropin (ACTH), luteinizing hormone (LH), prolactine (Prl), and growth hormone (Goya et al., 1994; Hadley et al., 1997; Brown et al., 1999). It was also shown that intracerebroventricular (i.c.v.) administration of thymulin modulates the serotonin neurotransmission in hypothalamic, mesencephalic, and striatal region of mouse brain (Vecsei et al., 1987). On the other hand, however, melatonin (produced in the pineal gland) is involved in the regulation of the level of thymic peptides: thymosin and thymulin in serum and thymus (Molinero et al., 2000). The 24 h rhythm of thymulin concentration in serum is probably evoked by melatonin action.

The regulatory activity of the CNS in respect to the secretory function of the thymus was also demonstrated by the observation that the young mice exposed to a noise show an increase of thymulin in the serum. At the same time the increase of thymus weight and the thymocyte number was observed (Folch et al., 1991).

The role of thymic peptides in the interactions between brain, thymus, and endocrine glands was earlier discussed by Martin-Du-Pan (1984). Among other publications concerning this problem a review of Bodey et al. (2000) may be indicated here.

The reciprocal interactions of the immune and neuroendocrine systems via thymic peptides on the one hand, and hormones derived from the hypothalamic-pituitary-adrenal axis on the other, were reviewed some years ago by Dardenne (1999) and Savino et al. (1999).

During the last years the evidence was collected on the direct effects of thymulin on the nervous system. In 1996 Safieh-Garabedian et al. found that i.p. injection of high doses of thymulin reduces significantly, and in a dose-dependent manner, the hyperalgesia evoked by intraplantar injection of the endotoxin from *Salmonella typhi* in the hind paw of rats. Further experiments showed a dual effect of thymulin as regards the pain phenomenon.

Whereas the high doses of thymulin reduce the inflammatory hyperalgesia, low doses of the hormone produce the hyperalgesia in experimental animals (Safieh-Garabedian et al., 1999). It was shown that the capsaicin sensitive primary afferents (CSPA) are involved in this effect of thymulin, which can indicate that the effect is mediated through the CSPA fibres (Saade et al., 1998).

The hypothesis that thymulin can affect central neurons either directly or through the peripheral nerve terminals was also supported by the finding that thymulin produces a significant and sustained fos-like-immunoreactivity in neurons located in spinal laminae known to be involved in nociception (Saade et al., 1999). However, in the opinion of the authors of the quoted paper, a prostaglandin E2-dependent mechanism which acts humorally on the CNS can be also involved in the thymulin-induced hyperalgesia.

It was found recently that thymulin reduces the hyperalgesia induced by cutaneous leishmaniasis in mice (Kanaan et al., 2002). It was also shown that pretreatment with thymulin i.c.v. injection of endotoxin reduces the endotoxin-induced hyperalgesia in rats in a dose-dependent manner (Safieh-Garabedian et al., 2003).

A potent peptide analogue of thymulin with a powerful inhibitory activity in respect to the pain of neurogenic origin was also found and investigated (Saade et al., 2003)

1.2.2 Thymopoietin

The hormone thymopoietin is a 49-peptide immunoregulator produced by the epithelial cells of thymus. It was isolated by the G. Goldstein group from the bovine thymi during the search for a natural agent which causes the symptoms of the myasthenia gravis disease (see: Krishna et al., 1980). The improved sequence of the human polypeptide, as determined by Goldstein's group is (Harris et al., 1994):

PEFLEDPSYLTKDKLKSELVANNVTLPAGEQRKDVY VQLYLQHLTARN.

It is of interest that this sequence could be considered as a multiplicate of a short 16-peptide fragment:

P	E	F	L	E	D	P	S	V	L	T	K	D	K	L	K
S	E	L	V	A	N	N	V	T	L	P	A	G	E	Q	R
K	D	V	Y	V	Q	L	Y	L	Q	H	L	T	A	R	N

(the same and similar residues are indicated by solid and broken boxes, respectively).

This can suggest that the gene for this 48-peptide has been developed by the multiplication of a short DNA sequence. Thymopoietin-like motifs appear in the molecules of many important regulatory proteins (Siemion et al., 1997). The biological role of thymopoietin consists mainly in the propagation of thymocyte differentiation, but it also affects the neuromuscular transmission (Basch and Goldstein, 1974).

Pentapeptide thymopentin (RKDVY), a 32–36 fragment of thymopoietin, reproduces the biological effects of thymopoietin in many assay systems (Goldstein et al., 1979). In 1990 Klusa et al. found that thymopentin (TP-5) may have a stress protective effect in rodents (Klusa et al., 1990). Similar observations were made by Iurato et al. (1993) who concluded that TP-5 influences the behavior in rodents and, in particular, is capable of increasing the resistance to stressful stimuli and pain. In agoraphobic patients with phagocytic dysfunctions TP-5 partially restores immunological functions. However, a further depression of phagocytic activities occurs in coincidence with panic attack (Covelli et al., 1991)

Afterthen Vazhnycha et al. (1994) found that TP-5 exerts an adaptive effect in chronic stress in rats. The same research group also investigated the effect of TP-5 on the acute stress in rats and demonstrated the stress-protective effect of pretreatment with TP-5 in rats with various types of nervous regulation (Tarasenko et al., 1997, 2000).

The evidence on the presence of thymopoietin and thymopoietin/α-bungarotoxin/nicotinic receptors within the brain tissue, presented by Quik et al. (1991), was later retracted by Goldstein et al. (2000). It should be noted, however, that another research group found that the synthetic bovine thymopoietin inhibits the α-bungarotoxin binding to rat brain neural membranes (Jennings and Shieh, 1993).

In 1996 Menzaghi et al. reported that IRI-514, a synthetic analogue of TP-5 with the sequence Ac-Arg-Pro-Asp-Phe-NH_2, has a long-lasting modulatory effect on behavioral response to social stress in rats, which suggests that the peptides of TP-5 family may play a role in modulating both behavioral and neuroendocrine responses to stress.

It was also evidenced by the electrophysiological method that TP-5 exerts the clear depressant neuromodulative effect upon skeletal neuromuscular synaptic activity *in vitro*. The isolated diaphragm of Wistar-strain rat was used in these experiments (Malicevic et al., 1994). It was also found that the prolonged subcutaneous treatment of a patient with multiple sclerosis with large doses of TP-5 results in a major improvement in his clinical condition (Borromei et al., 1995).

1.2.3 Thymosins

Thymosins were isolated from a fraction V (TFV) of a bovine thymic extract. In this fraction at least 30 peptides belonging to the α- and β-thymosins family were identified. The most important compounds of this mixture are thymosin α_1 (Thα_1) and thymosin β_4 (Thβ_4) that retain the major activity of TFV. Thymosin α_1, a 28-peptide, was extracted from calf thymus by Low et al. (1979).

Some sequential similarity exists between Thα_1 and thymopoietin, distinctly visible when the sequences of both bovine polypeptides are compared, which suggests that both peptides could have developed from the same ancestral gene:

The multiple action of thymosins on the immune, endocrine, and central nervous systems, as well as their potential clinical applications, were revised recently by Goldstein and Badamchian (2004).

Thα_1 is a potent immunostimulator, widely used in the diagnosis and treatment of many diseases (see: Ancell et al., 2001). It was found that Thα_1 is also expressed in specific neuronal populations of rats but its role within the brain tissue remains unclear (Turrini and Aloe, 1999). Recently its modulatory effect on the excitatory synaptic transmission in cultured hippocampal neurons was documented (Yang et al., 2003).

On the other hand, the concentration of both Thα_1 and thymulin in rat serum and thymus is regulated by melatonin. Melatonin regulates also the expression of the prothymosin alpha gene (Molinero et al., 2000). This documents once more the reciprocal molecular links, which exist between the immune and central nervous systems.

The evidence on the multiple biological functions of β-thymosin were lately reviewed by Huff et al. (2001). Thymosin β is an intracellular G-actin sequestering peptide and its main role consists in the regulation of actin polymerization. The sequence of thymosin β_4 was established by Low et al. (1981). It is as follows:
Ac-SDKPDMAEIEKFDKSKLKKTETQEKNPLPSKETI EQEKQAGES

Rebar et al. (1981) found that thymosin β_4 affects the hypothalamus and pituitary, stimulating secretion of the lutenizing hormone-releasing factor. However, the thymosins in the brain can also be of endocellular origin. In 1994 Zhang et al. found that Thβ_4, its close homologue Thβ_{10}, as well as Thα_1 are expressed in the brain tissue. This discovery strongly supported the supposition that thymosins may participate in the brain functions (Zhang et al., 1994). In fact, it follows from the investigations of Gomez-Marquez and Anadon (2002) that Thβ_4 and Thβ_{10} could play a specific function during cerebellum formation. Gomez-Marquez et al. (1996) found that thymosin β_4 is expressed in the murine central nervous system. The expression pattern of thymosin β_4 observed in the nervous system of mice demonstrates a high similarity to that found recently for the expression of Thβ_4 during chick development (Dathe and Brand-Saberi, 2004).

During the investigation of the development of intermediate-term memory in Hermissenda, Crow et al. (2003) showed that it is associated with the phosphorylation of the phosphoprotein Csp24. After cloning of the respective cDNA they found that this protein contains multiple β-thymosin-like domains. The influence of other β-thymosin-like repeat proteins on the phosphorylation of Csp24 was also examined (Crow et al., 2004).

1.2.4 Thymic humoral factor

Thymic humoral factor (THF-γ2) was identified by Trainin and Small (1970) as the factor conferring the immunocompetence on lymphoid cells. It is an octapeptide with the sequence: LEDGPKFL. The biological effects of THF-γ2 were reviewed by Cordero and Nogueira (1998).

To clarify the possible influence of immunization and the course of the immune response on the brain activity, Saphier et al. (1987a, b) employed an animal model bearing chronically implanted recording electrodes in the preoptic area/anterior hypothalamus (PO/AH) and hypothalamic paraventricular nucleus (PNV). Thymic nuclear factor was found to decrease multiunit electrical activity in the indicated areas of the brain, increased during immunization process (Saphier, 1989; Kidron et al., 1989; Saphier et al., 1990).

The possible therapeutic effects of THF-γ2 on pediatric patients with ataxia telangiectasia and Down's syndrome (Handzel et al., 1979) as well as on the patients with subacute sclerosing panencephalitis (SSPE) were investigated (Handzel et al., 1983).

2 Neuropeptides

2.1 Substance P and neurotensin

The first neuropeptide for which the activity in the immune system was reported was, according to our

knowledge, substance P (SP). Substance P is a nonapeptide belonging to the tachykinin family. It is present in the central and peripheral neural systems and, as it was suggested by Nicoll et al. (1980), plays a role of neurotransmitter. It is produced by several types of brain cells, including astrocytes, neurons, and microglia.

The amino acid sequence of substance P is as follows: Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

In 1980 Bar-Shavit et al. demonstrated that this peptide, as well as its N-terminal tetrapeptide fragment, are as active as tuftsin in phagocytosis stimulation and compete with tuftsin for its receptors on leukocyte cell membranes (Bar-Shavit et al., 1980).

However, in contrast to tuftsin, SP1-4 is ineffective in suppressing the morphine withdrawal syndromes (Aronowski et al., 1985).

As we have noted before (Siemion et al., 1990), tuftsin-like sequence Asn-Lys-Pro-Arg and a fragment Lys-Pro-Arg-Arg-Pro resembling the N-terminal tetrapeptide of substance P, appear in the sequence of neurotensin, tridecapeptide Glp-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu, which is present in the gut and brain tissues. Neurotensin, produced mostly in hypothalamus, possesses the activity typical for serum kinins.

As we have shown, neurotensin antagonizes the antinociceptive effects of enkephalins and potentiates the analgesic action of tuftsin (Siemion et al., 1990).

Hartung et al. (1986) investigated substance P binding properties in guinea pig macrophages. The contemporaneous study of Bar-Shavit and Goldman (1986) showed that two classes of binding sites for neurotensin are present on mouse macrophages, whereas macrophages have only one class of receptors for substance P. The highly efficient receptors for substance P were also found on mononuclear leucocytes (Bost, 1988).

For many years tachykinins were considered as peptides of neuronal origin. However, the recent works established the presence of tachykinins in other kinds of cells, among others in the cells of the immune system. As an interesting recent observation we can quote the results of Ho et al. (1997) on the expression of SP in human immune cells and its enhancement evoked by HIV (Ho et al., 2002). Thus, not only SP (and, possibly, other tachykinins) produced by peripheral neurons, but also produced by other cells, including leucocytes, may influence the immune response.

Substance P may regulate the inflammatory and immune response by influence on the production of inflammatory cytokines by human monocytes (Lotz et al., 1988). It induces the generation of IL-1-like activity in P388D1 cells (Kimball et al., 1988) and the enhancement of immunoglobulin secretion by B-lymphocytes, as well as the enhancement of natural killer activity in murine leukocytes (Croitoru et al., 2000).

The stimulation by SP of IL-1 expression by astrocytes was reported also by Martin et al. (1992). The peptide also strongly stimulates the production of IL-2 by splenic T-cells (Rameshwar et al., 1993). Gitter et al. (1994) evidenced that it also induces the production of IL-6 by human astrocytoma cells and Luber-Narod et al. (1994) found that it enhances the TNF-α production in neuroglia cells.

The astrocyte stimulation by SP to produce proinflammatory cytokines was reported by several groups (Wagner et al., 1987; Lotz et al., 1988; Blum et al., 1993; Lee et al., 1994). According to Bozic et al. (1996), SP acts as an amplifier of the inflammatory cascade in the immune complex-mediated injury.

Nilsson et al. (1987) investigated the influence of substance P on the proliferation of peripheral blood lymphocytes from normal individuals and birch pollen – allergic patients. They found that cells sampled from allergic patients show a more profoundly decreased response to concavalin A in the presence of 10^{-8} M SP, as compared with their cells sampled before season.

Soder and Hellstrom (1987) examined the effects of 15 neuropeptides on the human thymocyte, guinea pig T-lymphocyte and rat B-lymphocyte mitogenesis. It was found that whereas neurotensin exerted a dose-dependent stimulatory effect, substance P was inactive in all the tests applied. The influence of substance P on T-lymphocytes proliferation was reviewed by Payan (1989), and its role in immunoregulation by McGillis et al. (1987). From the new literature the reviews by Rameshwar (1997), Weinstock and Elliott (1998), and Bost (2004) should be noted.

2.2 Neurokinins

Since the year 1984, when a new peptide of the tachykinin family (substance K) was found by Nawa et al., several other peptides of this group were found in mammals, among others neurokinin A (NKA) and neurokinin B (NKB).

neurokinin A:
His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2

neurokinin B:
Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2

Substance K, as well as macropeptide gamma, are the N-terminally extended forms of NKA. The publications concerning these peptides and their cellular receptors were recently reviewed by Pennefather et al. (2004).

The neurokinin receptors are expressed by several types of immune cells: human circulating lymphocytes, murine T and B cells, human monocytes, guinea pig macrophages and others. Correspondingly, the peptides of tachykinin family, in particular neurokinins, may modulate many immunological functions of such cells, as cytokine release, immunoglobulin secretion, cellular chemotaxis, phagocytosis, and proliferation of T cells (for the review see: Eglezos et al., 1991).

Among others, the influence of substance P and neurokinin A on the hematopoiesis process in the bone marrow should be indicated (Kang et al., 2004).

The bone marrow is an important part of the immune system. It is innervated by the peptidergic nerve fibers which are tachykinin positive (Goto and Tanaka, 2002; Tabarowski et al., 1996).

The neurotransmitters, released from innervated fibers, are involved in communication between bone marrow cells and the neural system, regulating the hematopoiesis process. However, SP and NKA can be also produced by resident bone marrow cells, forming an independent source of these neurotransmitters (Rameshwar and Gascon, 1996).

SP and NKA influence also the skin immune system, participating in the modulation of skin inflamation and wound healing (Scholzen et al., 1998).

2.3 Enkephalins and endorphins

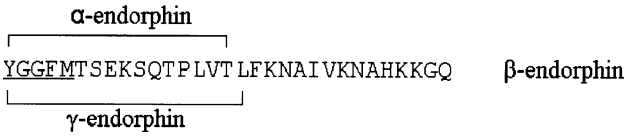
In 1975 Hughes et al. extracted two opioid pentapeptides from the pig brain: Leu-enkephalin and Met-enkephalin (Hughes, 1975). The peptides affect the opiate receptors in the tissues. Their interaction with receptors was found to be inhibited by naloxone.

Leu-enkephalin: Tyr-Gly-Gly-Phe-Leu

Met-enkephalin: Tyr-Gly-Gly-Phe-Met

Subsequently, the enkephalin-sensitive δ-receptors were identified in the cells by Lord et al. (1977). Except for the δ-receptors, two other types of opioid peptide receptors, μ and κ receptors, were also found.

At the same time, the research group of Guillemin discovered further opioid peptides with prolonged peptide chain, known as α-, β-, and γ-endorphins (Lazarus et al., 1976). The amino acid sequence of β-endorphin, which includes Met-enkephalin as well as α- and γ-endorphin, is as follows:



The half-life of both enkephalins in human plasma is about 5 min., and that of β-endorphin – nearly 40 min. (Venturelli et al., 1985; Martinez and Weinberger, 1988).

The earliest report concerning the effects of opioid peptides on the immune cells (human blood T lymphocytes) was presented by Wybran et al. (1979).

The growing knowledge on the immunomodulatory activity of enkephalins and endorphins was reviewed in the middle of the 80-ies by several authors (e.g. Teschemacher and Schweigerer, 1985; Wybran, 1985; Lewis et al., 1985).

In 1979 Wybran et al. suggested that μ and δ opioid peptide receptors should exist in peripheral blood T-lymphocytes. In fact, Leu-ENK receptors in cultured human T-lymphocytes were found by Ausiello and Roda (1984). The δ-receptors are involved in the stimulation of proliferation of human peripheral blood lymphocytes by Met-ENK; μ-agonists and antagonists were found to be inactive in this respect (Hucklebridge et al., 1989).

However, according to Taub et al., μ-selective agonists suppress the antibody production by the cultures of murine splenocytes (Taub et al., 1991). Carr et al. reported in 1989 that both δ and κ opioid receptors coexist in the same cells of T and B lymphocytes and macrophages.

A single κ-opioid binding site was found in a murine lymphoma cell line (Bidlack et al., 1992).

The κ receptors on thymoma cell line are down-regulated by κ-opioid agonists and this effect is reversed by naloxone (Joseph and Bidlack, 1995). The expression of opioid peptide receptors on the cells depends, however, on the proper status of the cells: only one type of low-affinity β-endorphin receptor was identified in unstimmulated mouse splenocytes by Jia et al. (1992), whereas concavalin A induces the expression of high-affinity receptors. The evidence concerning the opioid receptors in the cells of the immune system was reviewed by Sibinga and Goldenstein (1988) and Roda et al. (1996).

Among the opioid peptide receptors, specific for opiates (which could be inhibited by naloxone), the non-opiate receptors for β-endorphins were also found in the cultured human lymphocytes (Hazum et al., 1979).

In the opinion of Roda et al. (1996), opioid receptors seem to modulate immune functions in the prevalently

negative way, while the non-opioid receptors (to which the C-terminal fragment of β-endorphin binds) usually provide positive modulation.

However, the mechanism of neuroimmunomodulation by opioid peptides operates ''via complex mechanisms, involving multiple controls acting at different levels of integration: the level of a single cell, of the immune system, and at the level of the whole organism'' (Roda et al., 1996).

Correspondingly, α-endorphin and Met-enkephalin suppress the antibody secretion by mouse splenocytes (Johnson et al., 1982). The effect is antagonized by naloxone.

The suppression of the human PBMC antibody (PBMC – peripheral blood mononuclear cells) response by α-endorphin was reported by Heijnen et al. (1986).

Kusniecov et al. (1989) found that the effect of suppression of antibody secretion by α- and β-endorphins and Met-enkephalin in B-cells is also observed *in vivo*. The treatment of rats with Met- and Leu-enkephalin induced the decrease in anti-BSA antibody level (BSA – bovine serum albumin), mimicking to some extent the effects of electric tail shock on the humoral immune response to BSA (Stanojevic et al., 2003). The suppressive effect of β-endorphin on natural killer (NK) and cytotoxic activated T-lymphocytes was also found by Chiappelli et al. (1991).

However, positive modulation of antigen-induced human PBMC proliferation by enkephalin derived tetrapeptide Tyr-Gly-Gly-Phe (YGGF) was reported by Roscetti et al. (1988).

The up-regulation of mitogen-induced proliferation of splenic lymphocytes by β-endorphin was reported by Gilman et al. (1982). The effect, however, is realized by non-opioid mechanism.

It was also found by van der Bergh et al. (1993) that β-endorphin and its C-terminal fragments 6–31 and 18–31 enhance concavalin-A induced proliferation of rat T-splenocytes; opioid peptides with the intact N-terminal part were found to suppress this effect. It is also interesting that the enhancement of PHA-induced PBMC proliferation was also observed after low doses of the C-terminal dipeptide of β-endorphin, Gly-Gln (PHA - Phaseolus vulgaris agglutinin). However, the high doses of this dipeptide suppress the proliferation (McCain et al., 1987).

The β-endorphin receptors, resistant to naloxone (non-opioid receptors) can be, as it was indicated by Shahabi et al. (1991), inhibited by N-acetyl-β-endorphin. The indicated endorphin derivative suppresses PHA-stimulated proliferation of murine splenocytes. In this context it should be noted that N-acetyl-β-endorphin, and also β-endorphin 6–31 can displace β-endorphin bound to the same cells (Shaker et al., 1994).

The evidence quoted above seems to support the general conclusion given by Roda et al. (1996). However, a different picture results from the experiments concerning chemotaxis of immune cells. β-Endorphin and Met-enkephalin stimulate human PBMC chemotaxis via naloxone sensitive receptors (van Epps and Saland, 1984). The chemotaxis of neutrophils across endothelial monolayers is also stimulated by β-endorphin via opioid receptors (Wiedermann et al., 1994).

The peptides of enkephalin-endorphin group are also involved in the regulation of cytokine production. IL-2 and IL-4 production is stimulated in a dose-dependent manner in murine $CD4^+$ cells by α-, β-, and β-(6–31)-endorphins, as well as by opioid antagonists (van der Bergh, 1994).

β- and γ-Endorphins, as well as both enkephalins enhance the IL-1 production by a lymphoid cell line. The effect is realized via opioid receptors (Bessler et al., 1990). β-Endorphin and Met-enkephalin increase the secretion of interferon-γ by concavalin-A - stimulated human mononuclear cells (Brown and Van Epps, 1986). The same peptide modulates also the intracellular cAMP content in human peripheral blood mononuclear cells. However, whereas the peptide increases the cAMP concentration in cells with low baseline levels, it decreases it in cells with high baseline levels (Kavelaars et al., 1990).

Such dual modulatory effects are also observed in other phenomena mediated by opioid peptides. As it was reported by Rowland et al. (1987, 1989), Met-enkephalin suppresses the PFC in the splenocyte cultures in the presence of strong immune response. The suppression is, however, overcome by a weak immune response. Met-enkephalin enhances DTH inflammatory response in hairless guinea pigs at low doses (when injected together with elicitor), and suppresses it at higher doses (Sizemore et al., 2004). Dual modulation of mouse splenocyte was also reported by Gabrilovac (1993).

Phagocytic activity of peritoneal macrophages also decreases by *in vivo* treatment of naive mice with Met-enkephalin, and increases in mice immunized with SRBC (sheep red blood cells) (Marotti et al., 1993). Met-enkephalin and β-endorphin stimulate also in a dose-dependent manner the activity of neutrophils, evoking the increase in δ-receptor expression and up to 40% increase in the oxidative burst activity (Menzebach et al., 2003).

It was hypothesized in 1980 by Blalock and Smith that the lymphoid cells can produce the precursor of

endorphins. It is now evidenced that the β-endorphin produced by the cells of the immune system can alleviate intrinsic pain in the inflamed tissues, playing an important role in antinociception (Przewlocki et al., 1992). Recently Machelska et al. (2003) clarified the mechanisms of intrinsic pain inhibition in early and late inflammation. The opioid-containing leucocytes can migrate to the inflamed tissue affecting the opioid receptors on sensory nerves. In the early inflammation leukocyte-derived β-endorphin, Met-enkephalin, as well as dynorphin A interact with μ-, δ-, and γ-opioid receptors, inhibiting nociception. At a later stage the most prominent peptide involved is β-endorphin, acting at μ- and δ-receptors. The inflammatory processes may be also influenced by opiate alkaloids. Endogenous opiate alkaloids, naturally occurring in plasma, may interact with $\mu 3$ opiate specific receptors on inflammatory cells and constitute one of the immune inhibitory/antiinflammatory systems in the organism. On the other hand, the opioid peptides demonstrate the proinflammatory properties. This suggests some antagonistic action of opiates and opioid peptides in inflammatory processes (Stefano et al., 1996).

The presence of endogenous opiates in vertebrate tissues, including the nervous system, was demonstrated early by Gintzler et al. (1976, 1978). From the more recent works, papers of Donnerer et al. (1986) and Oka et al. (1985) could be mentioned.

The possible influence of endogenous morphinergic signaling on the tumor growth was discussed recently by Cadet et al. (2004).

Several immune cells produce preproenkephalin as the endogenous neuroendocrine hormone and cytokine. Such activity was evidenced for phagocytic cells (Vindrola et al., 1990), T-cells (Hook et al., 1999) and B-lymphocytes (Rosen et al., 1989). The importance of this neuroendocrine hormone for T-cell activation and proliferation was recently established by Hook et al. (2003).

Endogenous opioid peptides also stimulate cytotoxic activity of natural killer (NK) cells. The effect is realized via the δ-opioid receptors. It was shown that whereas δ-receptor specific agonist stimulates NK cells, δ-specific antagonists block the β-endorphin-stimulated NK function (Boyadjieva et al., 2001, 2002). In splenocytes, δ-opioid receptor expression is controlled by a negative feedback regulation of μ-opioid receptors. The opioid antagonist, naltrexone, disrupts this feedback control, reducing μ-opioid receptor functions (Boyadjieva et al., 2004), and thereby enhancing NK activity related to the μ-receptors.

The influence of Met-enkephalin on AIDS progression was documented by Gabrilovac and Marotti (2000), and Petersen et al. (2001). The proliferation effect of this peptide on the cultured lymphocytes infected by simian immunodeficiency virus (SIV) was reported by Hao et al. (2003). The promotion of the survival of SIV infected lymphocytes by Met-enkephalin provides, in the opinion of Li et al. (2004), a further evidence for the potential use of Met-enkephalin in AIDS therapy.

2.4 Other neuropeptides

Oxytocin and its transport protein neurophysin, were found in thymus extracts (Geenen et al., 1986). The presence of both oxytocin and vasopressin mRNA in the thymic tissue suggests the local synthesis of both these hormones (Geenen et al., 1987). They play a role of substitutes for IL-2 in the regulation of γ-interferon production by mouse splenocytes (Johnson and Torres, 1985).

Adrenocorticotropin (ACTH) and β-endorphin are co-released from the pituitary gland under stressful conditions (Guillemin et al., 1977). It was found that receptors for ACTH exist in immunocompetent cells and the hormone suppresses the functions of T-cells (Johnson et al., 1982). The N-terminal 14-peptide of ACTH, α-melanotropin (α-MSH, melanocyte stimulating hormone), proved to be a potent anti-inflammatory agent by antagonizing IL-1 (Dulaney et al., 1992). Neuropeptide Y (NPY) stimulates granulocytes and splenic NK cells in Lewis rats, suggesting the participation of the peptide in immunomodulation (Horsten et al., 1998).

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