



Original article

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**IMMUNOMODULATORY ACTIVITY OF
METHIONINE-ENKEPHALIN,
LEUCINE-ENKEPHALIN, INHIBITORS OF
ENKEPHALIN DEGRADING ENZYMES AND
"RELEASING" DIPEPTIDE AFTER
INTRACEREBROVENTRICULAR
ADMINISTRATION IN RATS**

SUMMARY

The aim of this work was to clarify the role of opioid peptides, inhibitors of enkephalin degrading enzymes and releasing dipeptide on humoral immune response after intracerebroventricular (i.c.v.) application in rats.

Methionine-enkephalin (Met-Enk) was i.c.v. injected through a permanently inserted cannula in various doses (0.01, 0.1, 1, 10, 20 and 50 µg/bw). Each rat received a total of 4 injections. Saline controls were treated in an identical manner. Met-Enk increased plaque forming cell assay (PFC response) in doses of 0.1 and 1.0 µg/bw, but in higher doses did not cause statistically significant suppression of PFC response. The immunopotentiating effects of Met-Enk were completely abolished by prior i.c.v. injection of ICI 174864 (delta opioid antagonist) in doses of 10 and 50 µg/bw.

The effects of Leucine-enkephalin (Leu-Enk) on PFC response were investigated by i.c.v. application of this peptide in doses of 0.1, 1, 10, 20 and 50 µg/bw. Treatments were performed in an identical manner as for Met-Enk. Leu-Enk caused statistically significant rise in PFC response when it was applied in doses of 0.1 and 1.0 µg/bw, whereas this substance in doses of 20 and 50 µg/bw exerted a suppression of the PFC response. The immunopotentiality, i.e. immunosuppression was abolished by prior i.c.v. injection of β-funaltrexamine (β-FNA) – delta opioid antagonist (1.0 µg/bw of β-FNA blocked the rise in PFC response caused by Leu-Enk, while 20 µg/bw of this antagonist blocked the suppression of PFC response caused by Leu-Enk).

(Des-Tyr)-Methionine enkephalin, inhibitor of endopeptidase and aminopeptidase, was i.c.v. applied in doses of 20 and 200 µg/bw. Each rat received a total of 5 injections. This inhibitor produced dose-dependent immunomodulation, i.e. in lower doses this substance induced rise in PFC response, whereas in higher doses suppression of immune response.

Bestatin, inhibitor of aminopeptidase, i.c.v. injected in an identical manner as the previous inhibitor, produced a rise in immune response in dose of 20 µg/bw, but statistically significant depression of PFC response in dose of 200 µg/bw.

N-carboxy-Phe-Leucine, a potent endopeptidase inhibitor, was i.c.v. injected in doses of 5 and 500 µg/bw. This substance caused dose-dependent immunomodulating effects: PFC response was potentiated when it was applied in a lower dose and suppressed in a higher dose.

Actinonin, inhibitor of all enzymes that hydrolyse enkephalins, i.c.v. administered in doses of 20 and 200 µg/bw caused dose-dependent effects on humoral immune response. Actinonin produced the potentiation of PFC response in lower doses, whereas the suppression in higher doses.

Kyotorphin, the "releasing" dipeptide, i.c.v. administered in the same way as previous inhibitors, induced immunopotentiality in dose of 5 µg/bw, whereas immunosuppression of PFC response in dose of 500 µg/bw.

Naltrexone, the antagonist of opioid receptors, completely blocked the immunomodulating effects of (Des-Tyr)-Methionine enkephalin (naltrexone was applied i.c.v. prior to this inhibitor).

These results suggest that opioid peptides, the inhibitors of opioid degrading enzymes and "releasing" dipeptide possess immunomodulatory activity. The immunological effects of Leu-Enk, enkephalinase-inhibitors and "releasing" dipeptide are dose-dependent phenomena.

Key words: opioid peptides, inhibitors of opioid degrading enzymes, "releasing" dipeptide, humoral immune response.

INTRODUCTION

Endogenous opioid peptides (Methionine-Enkephalin and Leucin-Enkephalin) are immunoregulatory substances. They act as lymphocyte activators, modulators of T-cell receptors, chemoattractants for monocytes and regulators of lymphocyte function. Met-Enk and Leu-Enk induced dose-dependent effects on immune response: high doses of these peptides suppressed immune response, while low doses potentiated the immune function after intraperitoneal or intracerebroventricular application. Alterations of immune responsiveness were much more pronounced after administration of Met-Enk and Leu-Enk into the cerebral cavity (1, 2). Similarly, the effect of intrahippocampal microinjection of enkephalin on cellular immune function and hippocampal IL-1 alpha gene expression was studied in rats. The results of this study suggested that intrahippocampal enkephalin might play an important role in neuro-immunomodulation by enhancing the inhibition of IL-1 alpha gene expression in hippocampal formation (3).

It is now well accepted that the immunomodulatory effects of Met-Enk and Leu-Enk are related to the interactions of these peptides with specific opioid receptors in the central nervous system (CNS). Results from various studies indicate that the immunomodulatory effects of opioids can be attributed to interactions with the mu-opioid receptor (4). Immunomodulatory activities of synthetic opioids take part via the mu-opioid receptor present on cells (5). Converging lines of evidence indicate that the opioid receptors expressed by immune cells are often the same or similar to the neuronal subtypes, particularly delta and kappa (6). Some studies have shown that human T-cells bear receptors for Met-Enk on their surface (7, 8). The immunomodulatory properties of a synthetic dimeric opioid peptide, biphalin and its analogs were investigated in various *in vitro* tests. Biphalin and one of its analogues [Tyr-D-Ala-Gly-Phe-NH₂-NH-Phe(p-Cl)-H] stimulate human T cell proliferation, natural killer (NK) cell cytotoxicity *in vitro* and interleukin-2 (IL-2) production. Biphalin and its analogue also released chemokine like factor in the culture supernatant that was responsible for increased chemotaxis of monocytes. Furthermore, these peptides inhibited tumor necrosis factor (TNF-alpha) production in lipopolysaccharide (LPS) stimulated peripheral blood mononuclear cells (PBMC) and nitric oxide (NO) production in mouse macrophage cells (9). The precise cellular mechanisms underlying the immunomodulatory effects of opioids are largely unknown. In recent

years, investigations from several laboratories have indicated that opioids can operate as cytokines, the principal communication signals of the immune system. All of the major properties of cytokines are shared by opioids, i.e., production by immune cells with paracrine, autocrine, and endocrine sites of action, functional redundancy, pleiotropy and effects that are both dose- and time-dependent (10).

Enkephalins are rapidly degraded into inactive fragments by several peptidases. Tyr-Gly bond can be hydrolysed by membrane-bound aminopeptidases (11) named aminoenkephalinases. Enkephalins are easily metabolised by cleavage of the Gly-Phe bond under the action of peptidase originally designated "enkephalinase" – this is carboxy-enkephalinase (12). Furthermore, endoenkephalinase (dipeptidyl-aminopeptidase) can metabolise enkephalins on Gly-Gly bond releasing the Tyr-Gly fragment (13).

The inhibitors of enkephalin degrading enzymes are now available and these substances are able to prolong the duration of action of endogenously released or exogenously applied enkephalins. Owing to the role of enkephalins in immunomodulation, the enkephalin degrading enzyme inhibitors could occur also as new immunomodulating agents.

The possible explanation for immunomodulating effects of inhibitors of enkephalin degrading enzymes, except in one study (14), have not been investigated till today.

Therefore, the aim of the present work was to investigate the possible role of Met-Enk, Leu-Enk, inhibitors of enkephalin degrading enzymes and "releasing" dipeptides on humoral immune response determined by the "plaque-forming cell assay" (PFC response).

MATERIALS AND METHODS

Animals and surgery

Eight-week-old male Wistar rats, weighting 200–250g were used in the experiment. Animals were kept under standard housing conditions and given food and water *ad libitum*. The surgical procedure of cannulation was made between bregma and lambda, and polyethylene cannulae were inserted into the lateral brain ventricles (2mm lateral to the sagittal suture, 2mm caudal to the frontal suture, and 3.2 mm ventral to the surface of the cortex). The cannulae were then fixed to the skull using two metal screws and dental acrylic. Rats were maintained in individual cages and allowed to recover for 1 week prior to use in experiments. Experimental and control groups consisted of 15–30 animals.

Drugs

The following substances were used for treatment:

- Met-Enk (Serva, Heildebreg, Germany)
- Leu-Enk (Serva, Heildebreg, Germany)
- ICI 174864 (Serva, Heildebreg, Germany)
- β -Funaltrexamine (Serva, Heildebreg, Germany)
- (Des-Tyr¹)-Metenkephalin (Serva Feinbiochemica GmbH and Co., Heildeberg)
- Bestatin (GMA Chemie GmbH)
- N-Carboxy-Phe-Leucine (GMA Chemie GmbH)
- Actinonin (Sigma Chemie GmbH, Deisenhofen)
- Kyotorphin (Sigma Chemie GmbH, Deisenhofen)
- Naltrexone (Sigma Chemie GmbH, Deisenhofen)

Antigens and adjuvants

Fresh sheep red blood cells kept in Alsever's solution served as antigen for induction of PFC response.

Immunization and immune response

For direct PFC assay rats were intraperitoneally immunized with 1ml of 50% suspension of sheep red blood cells. On the day after immunization animals were sacrificed, spleens removed and minced through stainless steel mash. Splenocytes were washed three times, and single cell suspension from each rat adjusted to 1×10^7 cell/ml in Medium 199 (Flow Laboratories, UK). Suspensions containing 1×10^6 spleen cells, 1×10^8 sheep red blood cells and guinea pig serum, which served as a source of complement (diluted 1/10) were transferred to Cunningham chambers and incubated for 45 minutes at 37° C. The number of hemolytic plaques was counted under light microscope and expressed as the number of PFC/ 10^6 spleen cell per rat.

Treatment

The treatment schedule in experiments with Met-Enk and Leu-Enk was:

The first injection was given 1h before immunization with SRBC and then after 24, 48 and 72 hours. Each rat received a total of 4 i.c.v. injections. The day after the last injection the rats were sacrificed and PFC response was performed.

– Met-Enk was injected in following doses: 0.1, 1, 10, 20 and 50 μ g/bw.

– Leu-Enk was given in following doses: 0.1, 1, 10, 20 and 50 μ g/bw.

In antagonistic studies the substances were administered by a single injection 72hrs after immunization:

– 1 μ g/bw of ICI 174864 followed by i.c.v. injection of 1 μ g/bw of Met-Enk

– 10 μ g/bw of ICI 174864 followed by i.c.v. injection of 1 μ g/bw of Met-Enk

– 50 μ g/bw of ICI 174864 followed by i.c.v. injection of 1 μ g/bw of Met-Enk

– 0.5 μ g/bw of -FNA followed by i.c.v. injection of 1 μ g/bw of Leu-Enk

– 1 μ g/bw of -FNA followed by i.c.v. injection of 1 μ g/bw of Leu-Enk

– 20 μ g/bw of -FNA followed by i.c.v. injection of 20 μ g/bw of Leu-Enk

The treatment schedule in experiments with inhibitors of enkephalinases and "releasing" dipeptide was:

The first injection was given 1h before immunization with SRBC, and then after 24, 48, 72 and 96hrs. Each rat received a total of 5 i.c.v. injections. The day after the last injection the rats were sacrificed and PFC response was performed.

– (Des-Tyr¹)-Met-Enkephalin was injected in doses of 20 and 200 μ g/bw

– Bestatin was administered in doses of 20 and 200 μ g/bw

– N-Carboxy-Phe-Leucine was given in doses of 5 and 500 μ g/bw

– Actinonin was applied in doses of 20 and 200 μ g/bw

– Kyotorphin was injected in doses of 5 and 500 μ g/bw

In antagonistic studies with naltrexone the treatment schedule was: the first injection of naltrexone was injected 1h before immunization with SRBC and after 30 minutes the rats were treated with (Des-Tyr¹)-Metenkephalin. After 24, 48, 72 and 96 hrs the rats were i.c.v. treated with the same substances in an identical manner as the first time. Each rat received a total of 5 injections:

– 10 μ g/bw of naltrexone followed by i.c.v. injection of 20 μ g/bw of (Des-Tyr¹)-Metenkephalin

– 10 μ g/bw of naltrexone followed by i.c.v. injection of 200 μ g/bw of (Des-Tyr¹)-Metenkephalin

All mentioned groups of animals had control groups. These groups were i.c.v. treated with 6 l of saline in an identical manner as experimental groups. In antagonistic studies, each rat was treated with 12 l saline.

RESULTS

Modulation of PFC response after i.c.v. application of Met-Enk (figure 1).

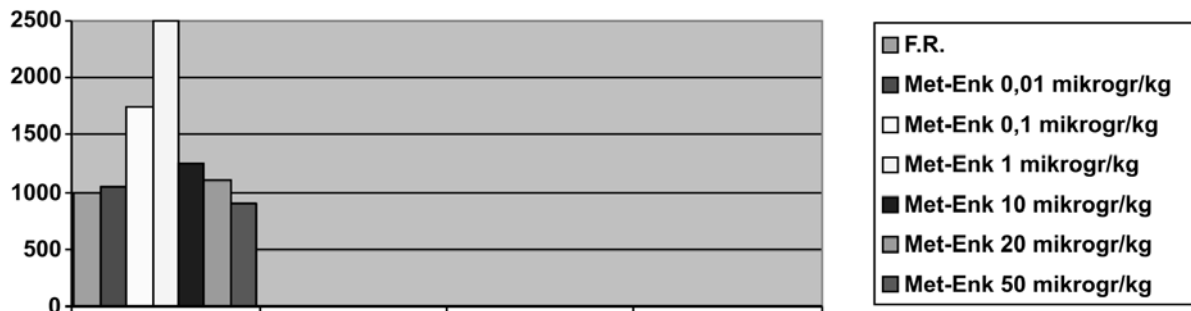


Figure 1. The potentiation of PFC response following i.c.v. administration of Met-Enk, starting from the day of immunization and then for three consecutive days. Statistically significant differences: $**p < 0.01$, rats treated with Met-Enk in doses of 0.1 and 1 $\mu\text{g}/\text{bw}$ vs. saline controls.

Antagonising effect of ICI 174864-delta opioid antagonist on stimulation of PFC response induced by i.c.v. injected Met-Enk (figure 2).

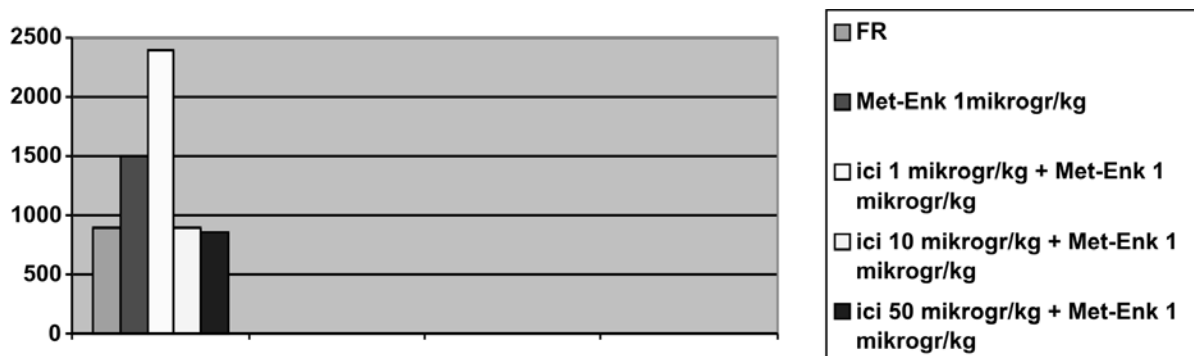


Figure 2. I. c. v. application of ICI 174864 in doses of 10 $\mu\text{g}/\text{bw}$ and 50 $\mu\text{g}/\text{bw}$ completely abolished stimulation of PFC response produced by i.c.v. administration of Met-Enk in dose of 1 $\mu\text{g}/\text{bw}$. Statistically significant differences: $***p < 0.001$, rats treated with Met-Enk (1 $\mu\text{g}/\text{bw}$) vs saline controls; $*p < 0.05$, rats i.c.v. treated with ICI 174864 (10 $\mu\text{g}/\text{bw}$) + Met-Enk (1 $\mu\text{g}/\text{bw}$) vs. rats treated with Met-Enk (1 $\mu\text{g}/\text{bw}$); $*p < 0.05$, rats i. c. v. treated with ICI 174864 (50 $\mu\text{g}/\text{bw}$) + Met-Enk (1 $\mu\text{g}/\text{bw}$) vs. rats i.c.v. treated with Met-Enk in dose of 1 $\mu\text{g}/\text{bw}$.

Modulation of PFC response after i.c.v. application of Leu-Enk (figure 3).

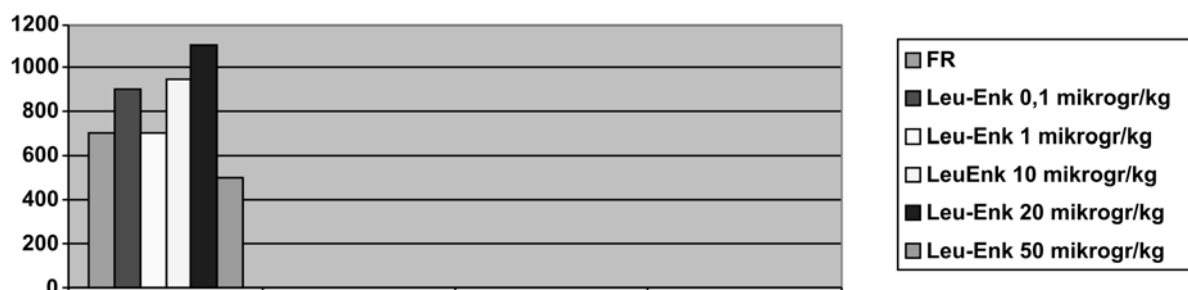


Figure 3. Dose-dependent stimulation and suppression of PFC response following i.c.v. administration of Leu-Enk, starting from the day of immunization and then for three consecutive days. Statistically significant differences: $*p < 0.05$, rats i.c.v. treated with Leu-Enk (1 $\mu\text{g}/\text{bw}$) vs. saline controls; $**p < 0.01$, rats i.c.v. treated with Leu-Enk (20 and 50 $\mu\text{g}/\text{bw}$) vs. saline controls.

Antagonizing effect of delta-opioid antagonist β -FNA on stimulation of PFC response induced by i.c.v. injected Leu-Enk (figure 4).

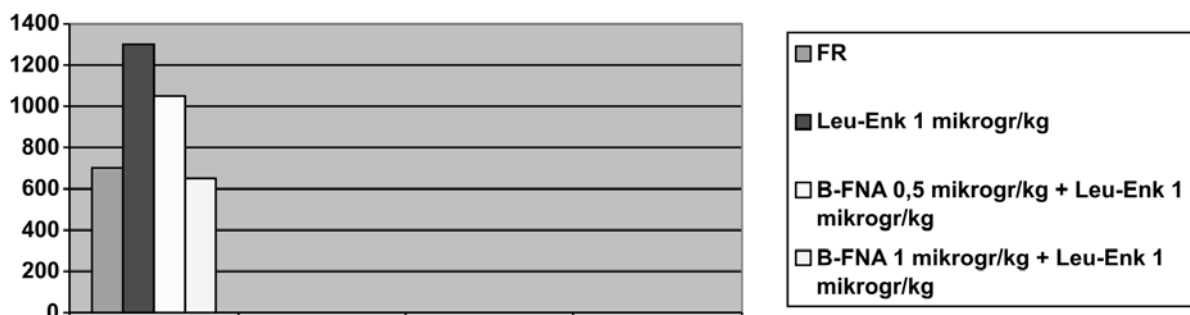


Figure 4. β -FNA i.c.v. administered in dose of 1 μ g/bw completely blocked the stimulation of PFC response produced by i.c.v. injection of Leu-Enk in dose of 1 μ g/bw.

Statistically significant differences: $**p < 0.01$, rats i.c.v. treated with Leu-Enk (1 μ g/bw) vs. saline controls; $*p < 0.05$, rats i.c.v. treated with β -FNA (0.5 μ g/bw) + Leu-Enk (1 μ g/bw) vs. rats i.c.v. treated with Leu-Enk (1 μ g/bw); $**p < 0.01$, rats i.c.v. treated with β -FNA (1 μ g/bw) + 1 μ g/bw of Leu-Enk vs. rats i.c.v. treated with 1 μ g/bw of Leu-Enk.

Antagonizing effect of β -opioid antagonist -FNA on suppression of PFC response induced by i.c.v. injected Leu-Enk (figure 5).

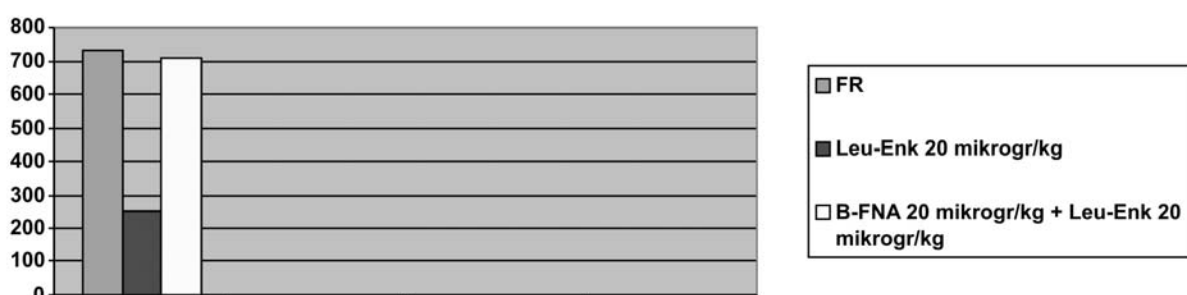


Figure 5. β -FNA i.c.v. administered in dose of 20 μ g/bw blocked the suppression of PFC response produced by i.c.v. injection of Leu-Enk in dose of 20 μ g/bw.

Statistically significant differences: $*p < 0.05$, rats i.c.v. treated with 20 μ g/bw of Leu-Enk vs. saline controls; $**p < 0.01$, rats i.c.v. treated with β -FNA (20 μ g/bw) + Leu-Enk (20 μ g/bw) vs. rats i.c.v. treated with Leu-Enk (20 μ g/bw).

Modulation of PFC response after i.c.v. application of (Des-Tyr¹)-Met-enkephalin (table 1).

Table 1. Dose-dependent stimulation and suppression of PFC response following i.c.v. administration of (Des-Tyr¹)-Met-enkephalin, starting from the day of immunization and then for four consecutive days. Statistically significant differences: $*p < 0.001$, rats i.c.v. treated with (Des-Tyr¹)-Met-enkephalin (20 μ g/bw) vs. saline controls; $*p < 0.01$, rats i.c.v. treated with (Des-Tyr¹)-Met-enkephalin (200 μ g/bw) vs. rats i.c.v. treated with saline.

GROUP	DOSE (μ g/kg tt)	PFC/10 ⁶ ±SD
Saline control		1057 ± 133
(Des-Tyr ¹)-Met-enkephalin	20	1612 ± 434*
(Des-Tyr ¹)-Met-enkephalin	200	368 ± 396*

Number of rats in group: 20; $*p < 0.001$

Modulation of PFC response after i.c.v. application of Bestatin (table 2).

Table 2. Dose-dependent stimulation and suppression of PFC response following i.c.v. administration of Bestatin, starting from the day of immunization and then for four consecutive days.

GROUP	DOSE ($\mu\text{g}/\text{kg tt}$)	PFC/ $10^6 \pm \text{SD}$
Saline control		1057 \pm 133
Bestatin	20	1509 \pm 501*
Bestatin	200	321 \pm 234*

Number of rats in group: 20; * $p < 0.001$

Statistically significant differences: * $p < 0.001$, rats i.c.v. treated with Bestatin in dose of 20 $\mu\text{g}/\text{bw}$, vs. saline controls; * $p < 0.001$, rats i.c.v. treated with Bestatin in dose of 200 $\mu\text{g}/\text{bw}$ vs. rats i.c.v. treated with saline.

Modulation of PFC response after i.c.v. application of N-Carboxy-Phe-Leucin (table 3).

Table 3. Dose-dependent stimulation and suppression of PFC response following i.c.v. administration of N-Carboxy-Phe-Leu, starting from the day of immunization and then for four consecutive days.

GROUP	DOSE ($\mu\text{g}/\text{kg tt}$)	PFC/ $10^6 \pm \text{SD}$
Saline control		1057 \pm 133
N-Carboxy-Phe-Leu	5	2808 \pm 609
N-Carboxy-Phe-Leu	500	310 \pm 62

Number of rats in group: 20; * $p < 0.001$

Statistically significant differences: * $p < 0.05$, rats i.c.v. treated with N-Carboxy-Phe-Leu (5 $\mu\text{g}/\text{bw}$), vs. saline controls; * $p < 0.001$, rats i.c.v. treated with N-Carboxy-Phe-Leu (500 $\mu\text{g}/\text{bw}$) vs. saline controls.

Modulation of PFC response after i.c.v. application of Actinonin (table 4).

Table 4. Dose-dependent stimulation and suppression of PFC response following i.c.v. administration of Actinonin, starting from the day of immunization and then for four consecutive days.

GROUP	DOSE ($\mu\text{g}/\text{kg tt}$)	PFC/ $10^6 \pm \text{SD}$
Saline control		1057 \pm 133
Actionin	20	3162 \pm 559*
Actionin	200	891 \pm 224**

Number of rats in group: 20; * $p < 0.001$; ** $p < 0.05$

Statistically significant differences: * $p < 0.001$, rats i.c.v. treated with Actinonin (20 $\mu\text{g}/\text{bw}$) vs. saline controls; * $p < 0.05$, rats i.c.v. treated with Actinonin (200 $\mu\text{g}/\text{bw}$) vs. saline controls.

Modulation of PFC response after i.c.v. application of Kyotorphin (table 5).

Table 5. Dose-dependent stimulation and suppression of PFC response following i.c.v. administration of Kyotorphin, starting from the day of immunization and then for four consecutive days.

GROUP	DOSE ($\mu\text{g}/\text{kg}$ tt)	PFC/ $10^6 \pm \text{SD}$
Saline control		1057 \pm 133
Kyotorphin	5	1589 \pm 478*
Kyotorphin	500	756 \pm 191*

* $p < 0.001$

Statistically significant differences: * $p < 0.001$, rats i.c.v. treated with Kyotorphin (5 $\mu\text{g}/\text{bw}$) vs. saline controls; * $p < 0.05$, rats i.c.v. treated with Kyotorphin (500 $\mu\text{g}/\text{bw}$) vs. saline controls.

Antagonizing effect of naltrexone – opioid antagonist on stimulation and suppression of PFC response induced by i.c.v. injected (Des-Tyr¹)-Met-enkephalin (table 6).

Table 6. I.c.v. application of naltrexone in doses of 10 $\mu\text{g}/\text{bw}$ completely abolished the stimulation of PFC response induced by prior i.c.v. injection of (Des-Tyr¹)-Met-enkephalin in dose of 20 $\mu\text{g}/\text{bw}$. Naltrexone i.c.v. injected in dose of 10 blocked the suppressive effect of (Des-Tyr¹)-Met-enkephalin (200 $\mu\text{g}/\text{bw}$) too.

GROUP	DOSE ($\mu\text{g}/\text{kg}$ tt)	PFC/ $10^6 \pm \text{SD}$
Saline control		882 \pm 286
(Des-Tyr ¹)-Met-enkephalin +Naltrexone	20 10	621 \pm 328*
(Des-Tyr ¹)-Met-enkephalin +Naltrexone	200 100	909 \pm 203*

Number of rats in the group: 20

DISCUSSION

It is now well accepted that the nervous system, directly or indirectly, regulates and modulates the function of the immune system. The anatomic innervation of the thymus and spleen (15) is the way of direct influence, while the effects of hormones, soluble factors (interleukin-1, interleukin-2) and endogenous neuropeptides as enkephalins and endorphines (16) are indirect ways how the CNS modulates immune functions. Some regions of the CNS (hypothalamus, hypophysis) secrete hormones that can influence immune functions.

Lymphocytes possess multiple receptors and react on various opioid hormones, neurotransmitters and mediators which are produced by many organs and tissues. Neurotransmitters such as enkephalins (Met-Enk and Leu-Enk) induce direct immunomodulatory effects in vivo (17). The action of opioids on various immune parameters is a result of interaction of these substances with specific opioid

receptors on immunocytes (18). Granulocytes and monocytes possess and receptors (19), T and B lymphocytes receptors (20), while receptors were found on cultured lymphocytes and thymoma cells (21).

Among the great number of opioids, it is proved for enkephalins that they exert immunomodulating effects. In our work we found that Met-Enk caused statistically significant immunopotentiality when it was applied in low doses. Some other authors (22) revealed that Met-Enk exerted dual, dose-dependent activity: large doses suppressed, whereas small doses enhanced the humoral immune response after i.c.v. application.

Taking into account that modulation of immune response may be mediated by several opioid receptors, antagonistic studies proved that selective antagonist of opioid receptors (ICI 174864) completely abolished the increase of PFC response evoked by Met-Enk. Thus, immunopotentiality caused by Met-Enk is a result of activation of opioid receptors in the brain.

Leu-Enk produced alterations of immune responsiveness after i.c.v. application: immune reactions were suppressed by high, while potentiated by low doses of this substance. β -FNA reversed both immunomodulatory effects caused by Leu-Enk, indicated that central actions of Leu-Enk on PFC response were mediated by brain opioid receptors. According to the neurotransmitter roles of Met-Enk and Leu-Enk, these peptides are quickly removed from synaptic cleft. Several peptidases are able to cleave the endogenous pentapeptides into inactive fragments. Tyr-Gly bond can be hydrolysed by several membrane-bound aminopeptidases (11). Furthermore, a dipeptidyl-aminopeptidase activity is also involved in enkephalin degradation (13). However, the role of this enzyme in enkephalin metabolism seems to be not very important at the synaptic level suggesting that high concentrations of dipeptidyl-aminopeptidase are involved in the regulation of enkephalin levels within the neuron terminals (1). Enkephalins are easily metabolized by cleavage of Gly³-Phe⁴ bond under the action of two enzymes: peptidase originally named enkephalinase (12) and angiotensin converting enzyme.

The inhibitors of these various peptidases are now available. It is proved that these substances are able to prolong the duration of action of the endogenously released enkephalins following nociceptive stimuli and behave as new analgetics. Owing to the probable role of enkephalins in emotional and behavioral controls, inhibitors of enkephalin degrading enzymes could occur also as new psychoactive agents. Thus, it is proved that puromycin, (Des-Tyr¹)-Met-enkephalin, N-Carboxy-Phe-Leucin and bestatin possess antinociceptive activity (23). Acetorphan, the inhibitor of aminopeptidase, modulates the behavior of rats after i.c.v. application (24), while thirphan (inhibitor of the same enzyme) caused central and peripheral cardiovascular effects (25). Actinonin, the inhibitor of all enzymes, produced significant analgetic effects, too (26). Inhibitors of enkephalin degrading enzymes is connected with the role of opioid peptides in stress (27). In stress, hypophysis secretes ACTH and β -endorphin, while suprarenal glands secrete opioid peptides and catecholamines. All these substances greatly influence the immune system. The fact that inhibitors of enkephalin degrading enzymes block the metabolism of enkephalins, is a good base for presumption that they prolong and hence augment the immunomodulating effects of Met-Enk and Leu-Enk.

According to the fact that effects of enkephalins on humoral immune response are produced via central opioid receptors, antagonistic studies with naltrexone i.c.v. applied prior to (Des-Tyr¹)-Met-enkephalin showed that this antagonist completely blocked the immunomodulating effects of (Des-Tyr¹)-Met-enkephalin.

In our experiments, kyotorphin ("releasing" dipeptide) produced dose-dependent actions on humoral immune response. The mechanism of immunomodulating effects of kyotorphin is the result of the fact that this substance is probably able to release enkephalins.

The overall results of this work implicated that inhibitors of enkephalin degrading enzymes and "releasing" dipeptide can be used as potential immunomodulating agents. Now it has been proven for bestatin that it is a drug of choice for treatment of some kind of tumors (14).

The exogenous opioid peptide morphine, has recently been shown to exert strong immunosuppressive activity. This finding may be relevant to the potential use of Met-enkephalin in adjuvant therapy for immunocompromised states, such as acquired immunodeficiency syndrome (AIDS) or malignancies (28).

In our work we revealed that all examined substances exerted the effects on humoral immune response. The inhibitors of enkephalin degrading enzymes caused dose-dependent immunomodulating effects: in low doses they produced the potentiation of PFC response, while in high doses the suppression of PFC response.

The mechanism of immunomodulation caused by inhibitors of enkephalin degrading enzymes is not easy to interpret. In fact, it is very difficult to give a sharp conclusion to which degree the enkephalinases are inhibited under the action of inhibitors of these peptidases. One of the difficulties in interpretation of these results is the fact that most of these inhibitors of enkephalinases are not specific only for enkephalinases. It is proved that they can block other enzymes which are not involved in metabolism of enkephalins. For example, neutral endopeptidase is a nonspecific enzyme because this substance can hydrolyse many biological active peptides (29), substance P (30), angiotensin II (31) and so on.

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IMUNOMODULATORNA AKTIVNOST METIONIN ENKEFALINA, LEUCIN ENKEFALINA, INHIBITORA ENZIMA KOJI RAZGRAĐUJU ENKEFALINE I "RELEASING" DIPEPTIDA POSLE INTRACEREBROVENTRIKULARNE PRIMENE U PACOVA

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SAŽETAK

Cilj ovog rada je bio da rasvetli ulogu opioidnih peptida, inhibitora enzima koji razgrađuju enkefaline i "releasing" dipeptida na humoralni imuni odgovor posle intracerebroventrikularne (i.c.v.) aplikacije u pacova.

Metionin-enkefalin (Met-Enk) je i.c.v. primenjen preko permanentno usađenih kanila u različitim dozama (0.01, 0.1, 1, 10, 20 i 50 µg/kgtt). Svaki pacov je primio ukupno 4 injekcija. Kontrolne grupe su primile fiziološki rastvor na identičan način. Met-Enk je izazvao porast broja hemolitičkih plaka (PFC odgovor) u dozama od 0.1 i 1 µg/kgtt, ali u višim dozama nije prouzrokovao statistički značajno smanjenje PFC odgovora. Imunopotencirajući efekti Met-Enk bili su kompletno blokirani prethodnom i.c.v. injekcijom ICI 174864 (delta opioidni antagonist) u dozama od 10 i 50 µg/kgtt.

Efekti Leucin enkefalina (Leu-Enk) na PFC odgovor ispitivani su i.c.v. aplikacijom ovog peptida u dozama od 0.1, 1, 10, 20 i 50 µg/kgtt. Tretmani su urađeni na isti način kao i za Met-Enk. Leu-Enk je prouzrokovao statistički značajan porast PFC odgovora kada je bio primenjen u dozama od 0.1 i 1 µg/kg tt, ali je ova supstanca u dozama od 20 i 50 µg/kg tt ispoljila supresivno dejstvo na PFC odgovor. Imunopotencijacija, odnosno, imunosupresija bila je sprečena prethodnom i.c.v. injekcijom betafunaltreksamina (β-FNA)-opioidnim antagonistom (1 µg/kgtt). β-FNA je blokirao porast PFC odgovora uzrokovanog Leu-Enk-om, dok je ovaj antagonist u dozi od 20 µg/kg tt sprečio smanjenje PFC odgovora izazvanog Leu-Enk-om.

(Des-Tyr¹)-Metioninenkefalin, inhibitor endopeptidaze i aminopeptidaze, primenjen je i.c.v. u dozama od 20 i 200 µg/kgtt. Svaki pacov je dobio pet injekcija. Ovaj inhibitor je izazvao dozno-zavisne imunomodulatorne efekte tj. u nižoj dozi došlo je do porasta PFC odgovora, a u višoj do supresije imunog odgovora.

Bestatin, inhibitor aminopeptidaze, i.c.v. primenjen na identičan način kao i prethodni inhibitor, prouzrokovao je porast imunog odgovora u dozi od 20 µg/kgtt, a statistički značajnu supresiju PFC odgovora u dozi od 200 g/kgtt.

N-Karboksifenilleucin, moćni inhibitor endopeptidaze, je i.c.v. aplikovan u dozama od 5 i 50 µg/kgtt. Ova supstancija je izazvala dozno-zavisne imunomodulatorne efekte: PFC odgovor je bio potenciran kada je primenjen u nižoj dozi, a suprimiran posle primene više doze.

Aktinonin, inhibitor svih enzima koji hidrolizuju enkefaline, i.c.v. aplikovan u dozama od 20 i 200 µg/kgtt izazvao je dozno-zavisne efekte na humoralni imuni odgovor. Aktinonin je ispoljio potencirajuće dejstvo kada je primenjen u nižoj dozi, a suprimirajuće kada je dat u višoj dozi.

Kjotorfin, dipeptid koji povećava oslobađanje enkefalina, i.c.v. primenjen kao i prethodni inhibitori, indukovao je imunopotencijaciju u dozi od 5 µg/kgtt, a supresiju PFC odgovora u dozi od 500 µg/kgtt.

Naltrekson, antagonist opioidnih receptora, kompletno je blokirao imunomodulatorne efekte (Des-Tyr¹)-Metioninenkefalina (naltrekson je i.c.v. primenjen pre ovog inhibitora).

Ovi rezultati sugerišu da opioidni peptidi, inhibitori enzima koji razlažu opioide i "releasing" dipeptid poseduju imunomodulatornu aktivnost. Imunološki efekti Leu-Enk, inhibitora enkefalinaza i "releasing" dipeptida je dozno-zavistan fenomen.

Ključne reči: opioidni peptidi, inhibitori enzima koji hidrolizuju opioidne peptide, "releasing" dipeptid, humoralni imuni odgovor