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THE IMMUNOMODULATING EFFECTS OF SPECIFIC OPIOID ANTAGONISTS AFTER THEIR INTRACEREBROVENTRICULAR APPLICATION

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Summary. *The role of central opioid receptor types in the modulation of humoral immune response was not investigated so far. Therefore, the aim of the present work was to investigate the possible role of these receptors in immunomodulation. For this purpose, male Wistar rats were intracerebroventricularly (icv) injected with opioid receptor specific antagonists. Control groups of rats were treated icv with physiological saline. Primary humoral immune response was determined by the "plaque-forming cell assay" (PFC response). ICI 174864, a selective δ opioid receptor antagonist, administered icv at doses of 0.1; 1; 10; 20 and 50 μg/kg bw caused a statistically significant immunosuppression. β-funaltrexamine (β-FNA), specific μ opioid receptor antagonist, applied icv produced a significant immunosuppression only at lower doses of 0.01 and 0.1 μg/kg. Nor-binaltorphimine (nor-BNI), a selective κ opioid receptor antagonist administered icv, produced significant immunopotentiation at all applied doses (0.1; 1; 10 and 50 μg/kg bw) except for the lowest dose 0.01 μg/kg bw. Quaternary naltrexone (QNTx), which is a μ opioid antagonist at lower doses, but a nonselective opioid antagonist at higher doses, caused statistically significant potentiation on PFC response only when it was given icv at doses of 1 and 10 μg/kg bw. The results indicated differential involvment of central opioid receptor subtypes in immunomodulation.*

Key words. *Immunomodulation, PFC response, opioid receptors, opioid antagonists*

Introduction

The present study was performed to resolve the role of brain opioid receptors in immune reactions. The immune system consists of a cellular repertoir, soluble cytokines and immunoglobulins which function in concern with facilitatory and inhibitory signals to maintain immunologic homeostasis. The contribution of the central nervous system (CNS) in immunoregulatory signalling has been well established (1).

Various pharmacological, immunological, histochemical and biochemical methods have demonstrated that opioid peptides and their specific receptors play an important role in the regulation of a variety of physiological processes in the organism (2). The opioid peptides may specifically bind to at least four types of opioid receptors: μ, δ, κ and ε, which are unevenly distributed in cells and tissues (3). The earlest report of opioid receptors and opioid effects on cells of the immune system was that of Wybran and colegaues (1979). In nanomolar concentrations opioids were shown to affect active rosetting of human T lymphocytes and this effect was stereospecifically reversed by naloxone (4).

Recognition of the multiplicity of opioid receptors led to development of radioligands relatively selective for a receptor type (5).

Antagonism by naloxone (or the closely related naltrexone) is an essential characteristic of opioid-receptor mediated effects. Naloxone has the greatest affinity for μ receptors, but at higher concentrations also blocks the other opioid receptor types.

The antagonistic potency of a drug depends on its receptor affinity and concentration in relation to the endogenous or exogenously applied agonists (6). Thus, the determination of the dose of an opioid receptor antagonist required for a functional effect is important in assesing the possible physiological relevance of endogenous opioids at target sites in vivo.

In the present study, dose-dependent immunomodulatory effects of opioid receptor type-specific antagonists were investigated.

Materials and Methods

2.1 Animals and surgery

Eight week-old, male Wistar rats weighting 200 –250 g were used in the experiments. Animals were kept under standard housing conditions and given food and water ad libitium. Insertion of cannulae into the lateral brain ventricles was performed under light Nembutal anesthesia at coordinates 2 mm behind the frontal suture and 2 mm lateral to the bregma. After surgery, rats were maintained in individual cages and allowed to recover for one week prior to immunization and treatment with opioid receptor antagonists. Experimental and control groups consisted of 17–20 rats.

2.2. Drugs

The following opioid antagonists were used for treatments:

- opioid receptor antagonist – ICI 174864 (Cambridge Research Chemicals, Cambridge, UK),
- μ opioid receptor antagonist – β-funaltrexamine (Serva, Heidelberg, Germany),
- κ opioid receptor antagonist – Nor-binaltorphimine (Serva, Heidelberg, Germany),
- QTNx nonselective opioid antagonist – quaternerny naltrexone methobromide, (Boehringer-Ingelheim, Ingelheim, Germany).

Appropriate drug concentrations were prepared daily from sterile frozen stock solutions and a total volume of 6 μl injected icv by a Hamilton microsyringe.

2.3. Immunization and immune response

Sheep red blood cells preserved in Alsever's solution served as antigen for induction of plaque-forming cell (PFC) response.

For direct PFC assay rats were intraperitoneally immunized with 1 ml of 50% suspension of sheep red blood cells. On day 4 after immunization, animals were sacrified, 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 cells/ml in Medium 199 (Flow Laboratories, UK). Suspension containing 1×10^6 spleen cells, 1×10^8 sheep red blood cells and guinea pig serum, as a source of complement (diluted 1/10) were transfered to Cunningham chambers and incubated for 45 min 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.

2.4. Treatments

The first drug injection was performed 1 h before immunization, and than every day until sacrifice. Each rat received a total 4 injections. The day after the last injection animals were sacrified, and PFC response was performed. This treatment shedule was established as the most effective, after a series of preliminary trials consisting of repeated (less effective) or single (ineffective) drug injections.

In experiments with δ-receptor antagonist ICI 174864 groups of animals were icv treated with this substance at the following doses: 0.01; 0.1; 1; 10; 20 and 50 μg/kg bw.

β-funaltrexamine, μ receptor antagonist was icv administered to the rats at doses of 0.01; 0.1; 1; 10; 20 and 50 μg/kg bw.

Nor-binaltorphimine, κ receptor antagonist, was icv applied to the animals at doses of 0.01; 0.1; 1; 10 and 50 μg/kg bw.

In experiments with quaternary naltrexone groups of animals were icv treated with doses of 0.01; 0.1; 1; 10; 20 and 50 μg/kg bw.

Control groups of rats were injected icv with saline in an identical manner as experimental groups.

Statistical analysis of the data was performed using one way ANOVA followed by Fisher's PLSD test. Data were presented as means ± SD.

Results

Modulation of PFC response by the δ opioid receptor antagonist ICI 174864

The icv injections of 0.01; 0.1; 1; 10; 20 and 50 μg/kg bw of ICI 174864 resulted in a marked decrease in the number of PFC, except at the lowest dose of the drug. The degree of suppression did not vary among the effecetive doses employed.

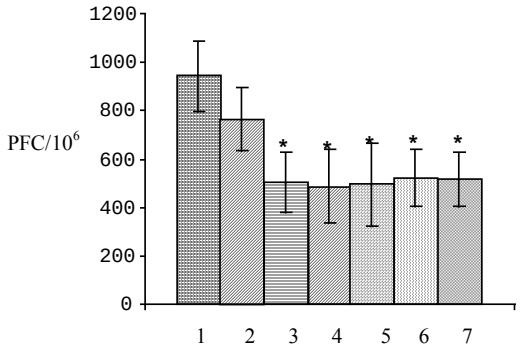

Fig. 1. Modulation of PFC response following icv treatment of rats with different doses of δ opioid antagonist ICI 174864.
(1–saline; 2–ICI in 0.01; 3–0.1 ; 4–1; 5–10; 6–20; 7–50 μg/kg bw)

Statistically significant differences: * $p < 0.05$ vs. saline controls.

Modulation of PFC response by the μ opioid receptor antagonist β-funaltrexamine

The icv injection of 0.01; 0.1; 1; 10 and 50 μg/kg bw μ opioid antagonist β-funeltrexamine resulted in a marked decrease in the number of PFC, but only when

the antagonist was administered at lower doses of 0.01 and 0.1 μg/kg bw.

Statistically significant differences: ** $p < 0.01$ vs. saline controls; *** $p < 0.001$ vs. saline controls.

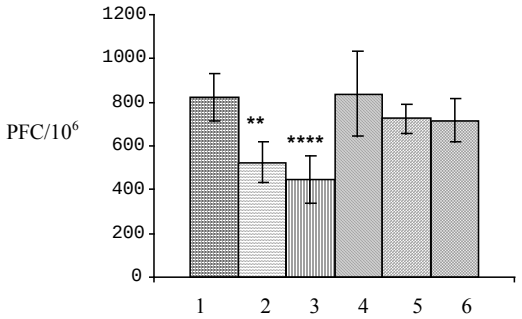

Fig. 2. Modulation of PFC response following icv treatment of rats with different doses of μ opioid receptor antagonist β-funaltrexamine. (1–saline; 2–β-FNA in 0,01; 3–0.1; 4–1; 5–10; 6–20; 7–50 μg/kg bw)

Modulation of PFC response by the κ opioid receptor antagonist Nor-binaltorphimine

The icv injections of 0.01; 0.1; 1; 10 and 50 μg/kg bw opioid antagonist Nor-binaltorphimine resulted in a marked increase in the number of PFC, except when the antagonist was applied at the lowest dose.

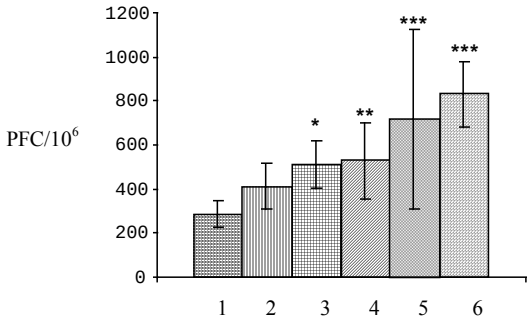

Fig. 3. Modulation of PFC response following icv treatment of rats with different doses of κ opioid receptor antagonist Nor-binaltorphimine. (1–saline; 2–nor-BNI in 0.01; 3–0,1; 4–1; 5–10; 6–20; 7–50 μg/kg bw)

Statistically significant differences: ** $p < 0.01$ vs. saline controls; *** $p < 0.001$ vs. saline controls.

Modulation of PFC response by quaternarny naltrexone

The icv injections of 0.01; 0.1; 1; 10; 20 and 50 μg/kg bw of quaternarny naltrexone resulted in marked increased in the number of PFC, but only when it was administered at doses of 1 and 10 μg/kg.

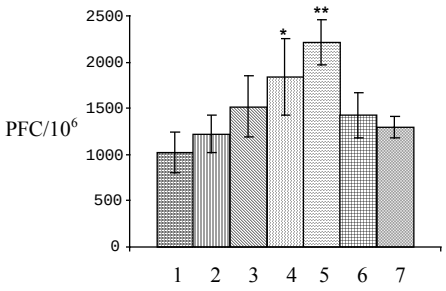

Fig. 4. Modulation of PFC response following icv treatment of rats with different doses of quaternarny naltrexone. (1–saline; 2–QNtx in 0.01; 3–0,1; 4–1; 5–10; 6–20; 7–50 μg/kg bw)

Statistically significant differences: * $p < 0.05$ vs. saline controls ** $p < 0.01$ vs. saline controls.

Discussion

The present study confirmed that endogenous opioid peptides and their specific receptors play an important role in regulation of immune functions.

The immunosuppressive effect of ICI 174864 on humoral immunity in the rats was most probably due to interference of this antagonist with endogenous δ opioid receptor acting peptides in the brain, thus implying that δ opioid receptors are involved in the stimulation of the immune response. Inside the central nervous system tissue, opioid peptides and receptors are present in neurons as well as in dinstinct populations of glial cells (7). In the hippocampus, these cell populations also secrete and respond to interleukine-1 (8) which interacts with opioid-binding sites (9), so introduction of an antagonist may affect glial-neuronal communication mediated by opioids and interleukins.

Most probably several neuro-endocrine axes are activated during immune reasponse and their functioning may be influenced by the opioid system. Thus, methionine-enkephalin (δ opioid receptor agonist) increased the release of growth hormone and prolactine (10) which potently stimulate immune response. Hence, ICI 174864 by blocking δ opioid receptors possibly diminished the release of growth hormone and prolactin.

β-funaltrexamine, a selective μ opioid receptor antagonist, is ireversibile antagonist of μ receptors, but reversible agonist of κ receptors (11). This antagonist caused a U-shaped dose-dependent suppression of humoral immune response and this effect probably include various mechanisms. In previous works (12) it was proved that methionine-enkephalin and leucine-enkephalin applied at low doses could cause immunopotentiation not only through δ receptors, but also through μ receptors. It is possible that the blockade of μ receptors by β-funaltrexamine inhibited the immu-

nostimulating tone which is delivered by μ receptors.

Moreover, Rothman et all. (13) proved that μ and δ receptors exist in alosteric interaction as integrative part of an receptor complex, and it is possible that β-funaltrexamine could antagonize δ agonistic interactions thus inhibiting the physological immunopotentiating tone mediated by δ opiod receptor ligands.

Immunosuppression caused by this antagonist can also be consequence of reversible stimulating influence of this substance on κ opioid receptors. Finally, the central immunosupprression evoked by β-funaltrexamine could be attributed to the ability of this antagonist to influence to various neuroendocrine axes, which are regulated by opioid peptides (14).

Nor-binaltorphimine, a selective κ opioid receptor antagonist, exerted a statistically significant increase of PFC response. Recently, it was proved that the κ opioid system in CNS had tonic immunosuppressive influence on immune functions (12), so nor-binaltorphimine probably interrupted this influence. The endogenous κ opioid receptor agonist (dynorphin) and κ opioid receptors are abundant in the hypothalamus. Stimulation of these receptors coud result in the observed immunosuppressive effects. In addition, it is proved that stimulation of κ opioid receptors by κ agonists can result in a decreased release of dopamine which exert immunopotentiation, so the application of nor-binaltorphimine may disrupt the dopaminergic influence on immune response (15).

The finding that quaternerny naltrexone, a nonselective antagonist, caused immunopotentiation appears, at first sight, paradoxical. The differential interactions among quaternerny naltrexone and multiple opioid receptors may be one of the reasons for the paradoxical effect of this substance reported here. Indeed, quaternerny naltrexone preferentially antagonize μ than κ and only at high doses δ receptors. Since, relatively small dose of quaternerny naltrexone was used for icv treatment, it is possible that this antagonist exerts its action by selectivelly blocking μ and/or κ receptors, inside the brain, thus increasing the endogenous δ opioid immuno-stimulatory tone. Another possibility would be that this antagonist may bind to a highly specific quaternarny naltrexone-binding sites in the rat brain, so the stimulation of this receptors may specifically mediate the agonist activity of quaternerny naltrexone.

In addition, the mechanisms involved in the central immunomodulatory actions of this antagonist may comprise a number of neuro-endocrine axes susceptible to and regulated by opioid peptides (14).

The results of this study indicated that specific opioid antagonist administered icv exert differential, receptor-type specific immunomodulatory effects. On the basis of our results, we can conclude the following:

1. ICI 174864 and β-funaltrexamine exert immunosuppressive effects.
2. Nor-binaltorphimine and quaternarny naltrexone produce immunopotentiation.

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IMUNOMODULATORNI EFEKTI SPECIFIČNIH ANTAGONISTA OPIOIDNIH RECEPTORA POSLE NJIHOVE APLIKACIJE U BOČNE KOMORE MOZGA

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Kratak sadržaj: *Uloga samostalno intracerebroventrikularno (icv) primenjenih antagonista opioidnih receptora na humoralni imuni odgovor do danas nije proučavana. Stoga je cilj ovog rada bio da rasvetli efekte ovih supstancija u imunomodulaciji. U tu svrhu koristili smo Vistar pacove, težine 200-250 g, koji su čuvani pod standarnim uslovima. Suptancije su icv aplikovane i sve grupe imale su kontrolne grupe koje su bile tretirane ekvimolarnim fiziološkim rastvorom. Imuni odgovor je odredjivan merenjem broja hemolitičkih plaka (PFC) odgovor. ICI 174864, selektivni delta opioidni antagonist, icv aplikovan u dozama od 0,1;1;10;20 i 50 μg/kg tt prouzrokovao je statistički značajnu imunosupresiju. Beta-funaltreksamin (beta-FNA), specifični mi opioidni antagonist icv aplikovan izazvao je statistički značajnu imunosupresiju, kada je bio primenjen u dozama 0,01 i 0,1 μg/kg tt. Nor-binaltorfimin (nor-BNI), selektivni kapa opioidni antagonist icv primenjen, prouzrokovao je statistički značajnu imunopotencijaciju u svim aplikovanim dozama (0,1; 1; 10 i 50 μg/kg tt), izuzev u najnižoj dozi (0,01 μg/kg tt). Kvaternerni naltrekson (QNTx) koji se ponaša kao mi opioidni antagonist u nižim dozama, a delta opioidni antagonist u višim dozama, ne prelazi krvno moždanu barijeru icv aplikovan izazvao je statistički značajnu potencijaciju PFC odgovora, ali samo kada je primenjen u dozama od 1 i 10 μg/kg tt.*

Ključne reči: *Imunomodulacija, PFC odgovor, opioidni receptori, opioidni antagonisti*

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