EFFECTS OF A NEWLY-SYNTHESIZED HEXAPEPTIDE ON PERIPHERAL ACUTE INFLAMMATION

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SUMMARY

Following the discovery of N/OFQ/NOP system, an intensive study was started to find selective ligands with presumptive therapeutic potential. Among the agonists, a hexapeptide Ac-RYYRWK-NH2 has been identified, expressing high NOP-receptor affinity and selectivity. Its molecule was used as a template, in which Trp^5 was substituted by original β^2-tryptophan analogue (*S*)-2-(1-methyl-1*H*-indol-3-yl)propionic residue. We found that the new compound (HP3) interacts with both NOP and opioid receptors. There is growing evidence for the involvement of N/OFQ/NOP system in inflammatory responses and sepsis. In addition, peripheral neurons and ectodermal cells also express opioid receptors, playing a critical role in modulating pain and inflammation. The aim of the present study was to examine the anti-inflammatory effect of the newly-synthesized hexapeptide HP3, which display affinity to both NOP and opioid receptors. In rats, peripheral inflammation was induced by carrageenan (CG) and the effects of 3 doses HP3 (10 μg/kg, 20 μg/kg and 40 μg/kg, i.p.), injected 15 min before CG, were examined. The volume of the injected paw was measured plethysmographically each 60 min for a period of 4 hours after CG- treatment and compared to the control group. We found, that 4 hours after CG injection the edema rate of the inflammated paw in all 3 doses HP3 tested was markedly lower, compared to that in CG-treated group Similar results have also been obtained when the animals in the experimental groups were injected with JTC-801 (1mg/kg). These experimental data give us ground to suggest that the newly synthesized hexapeptide PH3, injected 15 min before CG, exerts anti-inflammatory action, activating both opioid and NOP receptors.

Key words *inflammation, nociceptin, opioids, hexapeptides*

INTRODUCTION

The numerous endogenous opioid peptides (so called classical opioids endomorphines, enkephalins, etc.) exert their effects by activating *μ, δ, κ* and *ε* receptors. The hexadecapeptide nociceptin (N/OFQ) also belongs to the same opioid family but it does not interact with the classical μ, δ and κ receptors. [3, 6] Its structure, as well as the structure of its receptor (NOP), are very similar to those of the other opioid peptides. However, functional studies have shown that N/OFQ/NOP system is physiologically and pharmacologycaly independent.

It has long been known that opioids, especially peripherally acting, have anti-inflammatory effects. [10] Except the central nerve system, opioid receptors are widely spread in peripheral neurons, neuroendocrine immune and ectodermal sells [7]. These peripheral opioid receptors play a critical role in modulating pain and inflammation. Furthermore, opioids exert anti-inflammatory effects via changes in release of pro-inflammatory neuropeptides (SP, CGRP) [4, 11]. Recently, growing evidence appeared about involvement of the N/OFQ/NOP system in sepsis and inflammatory response [8]. Elevated plasma levels of N/OFQ are found in patients with Wilson disease [1], hepatocellular carcinoma [9], and in synovial fluids from arthritic patients [13]. There are a lot of studies, showing that N/OFQ acts as immunomodulator. Its effects on immune cells are mainly pro-inflammatory [8]. On the opposite, it inhibits the release of inflammatory mediators {substance P (SP) and calcitonin gene related peptide (CGRP) [2] and bradykinin [5]} from

peripheral nerve terminals, plateles, and mast cells. There are also data, showing that N/OFQ decreases carrageenan (CG)-induced paw-edema volume [12].

In our previous work, new ligands for NOP receptor have been synthesized, based on the NOP-agonist molecule Ac-Arg-Tyr-Tyr-Arg-Tyr-Lys NH_2 (Ac-RYYRWK-NH_2). Structure-activity study has shown that the substitution of Tyr^5 by original β^2-tryptophan analogue (*S*)-2-(1-methyl-1*H*-indol-3-yl)propionic residue (compound HP3) modified the selectivity of the peptide - the new compound interacts with both NOP- and opioid-receptors. Based on the fact, that activation of the both types of receptors influences the inflammatory process, we examined the effect of HP3 on acute CG-induced inflammation.

Material and methods

Synthesis

Using SPPS by Fmac chemistry and Ac-RYYRWK-NH_2 like a template, a series of new compounds were synthesized, in which Trp^5 was substituted by original β^2-tryptophan analogue (*S*)-2-(1-methyl-1*H*-indol-3-yl)propionic residue.

Biological activity

The biological activity of the newly synthesized analogs of the hexapeptide AcRYYRWK-NH2 was tested in vitro on electrically stimulated rat vas deferens (rvd) preparations. Prostatic segments of the smooth muscles, 12-15 mm long were fixed in organ bath with aerated Krebs solution and stimulated electrically with parameters 0.05 Hz, 1ms pulse duration, SMV. The evoked contractions were registered isometrically by on-line system. The tested compound was cumulatively applied. In some experiments, naloxone (Nal, $1x10^{-6}$M, as a blocker of opioid receptors) or naloxone benzoylhydrazone ($3x10^{-5}$M, as a blocker of opioid and NOP receptors) was administrated to the organ bath 10 min or 15 min prior to the peptides under investigation.

The experiments were performed according to the rules of the Ethic Committee of the Institute of Neurobiology, BAS.

Carrageenan (CG)-induced inflammation

The acute inflammation was induced by intraplantar (i.pl.) injection of 0.1 ml CG (1%, w/v) into the right hind paw. HP3 (10 μg/ml, 20 μg/ml, or 40 μg/ml; 0.1 ml/100g) was administered 15 min before the induction of the CG-inflammation. The paw volume was measured plethysmographically before the injection of CG to obtain a control value. After CG injection (served as "zero time"), the paw-edema volume was measured every 60 min for a period of 4 hours. Data are expressed as the edema rate (%). Values for each group represent the mean +SEM of 8-10 animals.

Experimental groups

The following experimental groups were created:

Control group – injected with CG and saline;

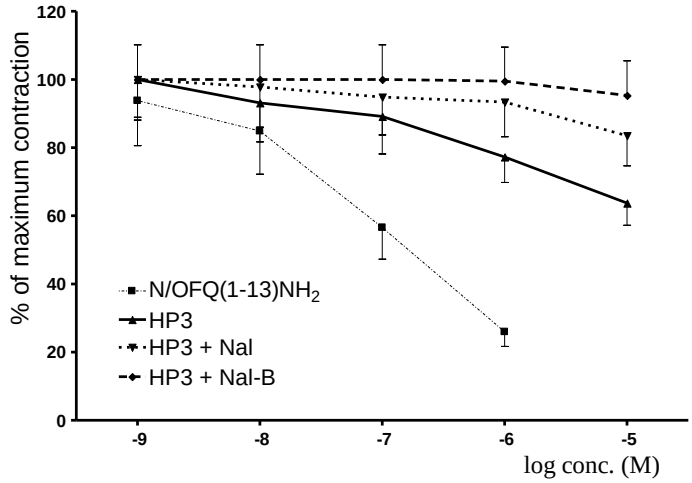
Experimental groups 1, 2 and 3 - injected with CG and HP3 10 μg/kg, 20 μg/kg, and 40 μg/kg, respectively; experimental groups 4, 5 – injected with Nal 1mg/kg, CG and HP3 20 μg/kg or HP3 40 μg/kg; experimental group 6, 7 - injected with JTC-801 1mg/kg, CG and HP3 20 μg/kg or HP3 40 μg/kg.

Nal (1mg/kg) was applied to block opioid receptors and JTC-801 1mg/kg was applied to block NOP receptors. Both inhibitors were injected 30 min before CG.

RESULTS AND CONCLUSIONS

Biological activity of HP3

HP3, in which Trp in position 5 is substituted by (*S*)-2-(1-methyl-1*H*-indol-3-yl)propionic residue, exhibited lower affinity (pEC_{50}=6.5), compared to the parent molecule(RYYRWK). The maximal inhibitory effect (E_{max} = -36%), was produced by the peptide in a concentration of $1x10^{-5}$M. The effect of HP3 was strongly reduced by pre-incubation of smooth muscles with Nal and completely prevented by Nal-B (Fig. 1). It is evident, that inclusion of N-methyl β2-tryptophan analogue in position 5 results in a peptide (HP3) that possesses affinity for both opioid- and NOP receptors.



Fugure 1. Rat vas deferens. Concentration-response curve of **HP 3** applied alone, and after naloxone or naloxone benzoylhydrazone, on the contractions induced by ES. The data are means ± SEM of six experiments.

During the first 120 min after CG injection, HP3 in all 3 doses used did not significantly change the paw edema volume. The edema rate was similar to that of the controls (Fig. 1). The first significant difference between the control and the experimental group injected with 40 μg/kg HP3 was found 180 min after CG-induced inflammation. At the end of observation (4 hours after CG injection) the edema volume of the inflamed paw was markedly lower in all 3 doses tested, compared to that in CG-treated animals (Fig 2).

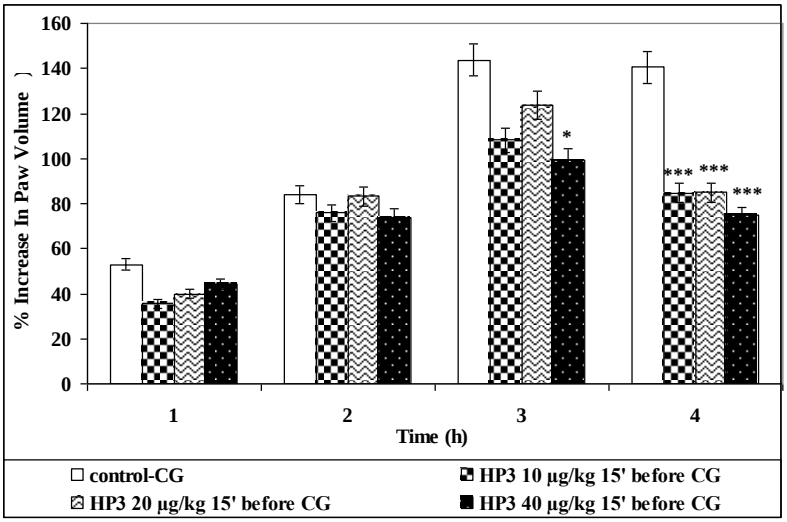


Fig.2. Effects of HP3 10 μg/kg, HP3 20 μg/kg and HP3 40 μg/kg on CG-induced acute inflammation. Statistically significant differences vs. controls at *p<0.05; ***p<0.001.

The pre-treatment of the animals with the opioid receptor antagonist Nal (1 mg/kg, i.p.) did not change the dynamic of peptide's anti-inflammatory action. The decrease in paw edema is well expressed, but not statistically significant (Fig. 3), showing that after inhibition of the classical opioid receptors HP3 exerts a weaker effect on the inflammation.

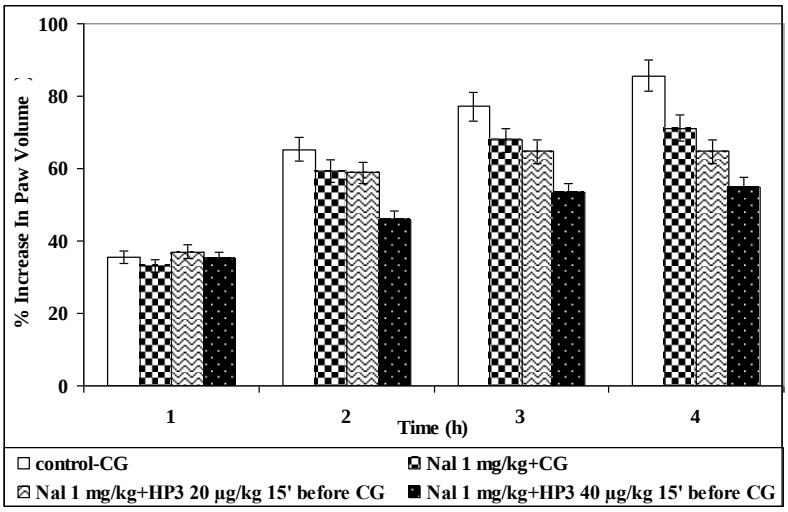


Fig.3 Effects of HP3 20 µg/kg and HP3 40 µg/kg on CG-induced acute inflammation after pre- treatement of the animals in the experimental groups with Nal (1mg/kg).

Similar results have also been obtained when the animals in the experimental groups were injected with JTC-801 (1 mg/kg) 15 min before HP3 (Fig 4).

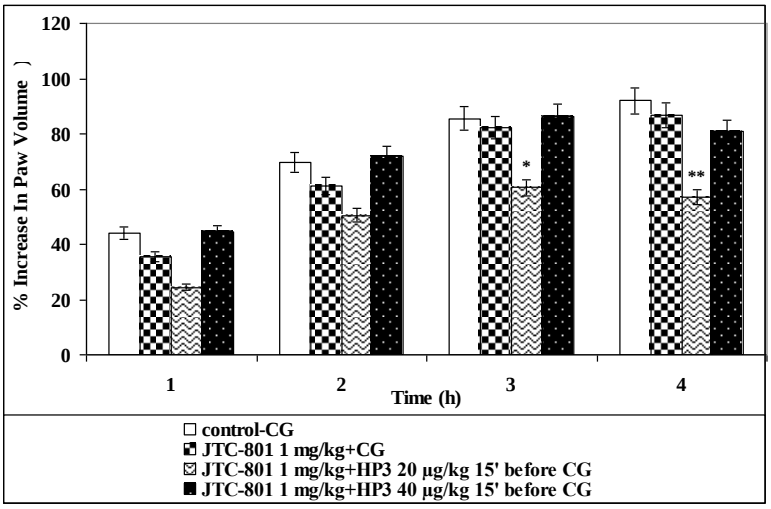


Fig.4. Effects of HP3 20 µg/kg and HP3 40 µg/kg on CG-induced acute inflammation after pre- treatment of the animals in the experimental groups with JTC-801(1 mg/kg); *p<0.05, **p<0.01.

It is interesting to note that, under these experimental conditions, HP3 20 µg/kg exerted much stronger anti-inflammatory effect, compared to the group, injected with the peptide only, suggesting that in this dose HP3 most probably activates predominantly opioid receptors. The effect of the higher dose used (40 µg/kg) does not statistically differ from the controls.

The experimental data obtained give us ground to suggest that, injected 15 min before CG, the newly synthesized hexapeptide PH3 exerts anti-inflammatory action, activating both opioid and NOP receptors.

Acknowledgements

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