IN Volvement of Vanilloid- and Cannabinoid Receptors in the Anti-Inflammatory Action of Nociceptin

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Abstract

In the present study, we have examined the probable interactions of N/OFQ(1-13)NH₂ and its structural analogue [Orn⁹]N/OFQ(1-13)NH₂ with cannabinoid CB₁-receptors on acute carrageenan (CG)-induced inflammation in rat paw, as well as the mechanism of these interactions. The study also aims to find out whether the TRPV1-receptors (transient receptor potential vanilloid 1) take part in these processes. Our results showed that the simultaneous treatment of rats with CB₁-receptor agonist HU-210 and the investigated peptides did not change the specific effects of the nociceptin and [Orn⁹]N/OFQ(1-13)NH₂. Applied after blockade of CB₁-receptors, the peptides did not exert their anti-inflammatory effects. On the contrary, when the TR PV1-receptors were blocked, the anti-inflammatory effects of nociceptin (NOP)-receptor agonists as a whole remained unchanged.

In conclusion, based on the results obtained, it might be suggested that N/OFQ(1-13)NH₂ and [Orn⁹]N/OFQ(1-13)NH₂ influenced the peripheral inflammation by interactions with their own NOP-receptors located on the primary sensory neurons.

We also suppose that there is a functional link between NOP- and cannabinoid CB₁-receptors. It might be assumed that vacant or activated CB₁-receptors are required for NOP-evoked inhibition of acute peripheral inflammation.

Key words: nociceptin, rat-paw inflammation, CB₁-receptors

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Introduction. In spite of the various investigations performed over the last years, the pathophysiology of acute peripheral inflammation still remains unclear. This interest is based on the fact that the inflammation usually accompanies many widespread diseases such as asthma, arthritis, colitis, gastritis, etc. The inflammation process is associated with release of many pro-inflammatory mediators by the cutaneous nerve endings, such as substance P (SP), calcitonin gene-related peptide (CGRP), neurokinin A, [1] usually followed by vasodilatation and increased vascular permeability, plasma extravasation, neutrophil infiltration, oedema, etc., in the area innervated by the stimulated nerve [2]. It has been found that nociceptive primary afferent neurons express both receptor types – NOP and CB1. Many data have shown that cannabinoids [3, 4] as well as nociceptin (N/OFQ) [5] reduce acute peripheral inflammation by suppressing the release of substance P and CGPR from the primary sensory neurons. Generally, cannabinoids reduce inflammation and hyperalgesia by activating the peripheral CB1-receptor. The effects of some CB1-agonists are mediated via the TRPV1-receptor [6, 7]. On the other hand, it is worth mentioning that N/OFQ inhibits the effects of the activated TRPV1-receptors [8, 9, 5].

Taken together, literature data suggest common stages in the mechanisms of action of cannabinoids and nociceptin in inflamed tissues and also that the involvement of vanilloid receptors in them is very likely. Therefore, compounds affecting the activity of TRPV1-receptors (cannabinoids, nociceptin) may have therapeutic usefulness in a number of disorders, including pain and inflammation.

Now, in our study we aimed to investigate the presumed hypothesis for a functional link between the two systems, testing the effects of cannabinoids and nociceptin (applied alone, or combined, or after inhibition of vanilloid receptors) on acute carrageenan-induced inflammation.

Materials and methods. The experiments were performed on male Wistar rats weighing 180–200 g and housed at 22–25 °C. The animals were allowed an acclimatization period, with free access to food and water, and a natural day/night light cycle.

The acute inflammation was induced by intraplantar injection (i.pl.) into the right hind paw, in a volume of 0.1 ml carrageenan, which provoked oedema. The carrageenan model is appropriate and widely used for testing the anti-inflammatory activity of different substances, including drugs [10].

The volume of paw-oedema was measured with a plethysmometer (Ugo Basile, Varese, Italy) every 60 min, for a period of 4 h after CG injection. The moment of CG injection serves as time zero. To obtain a control, the right hind paw was also measured before the CG injection. Data are expressed as changes in the oedema (difference from the pre-CG volume).

N/OFQ(1−13)NH₂ and [Orn9N/OFQ(1−13)NH₂(20 µg/kg)

were intraperitoneally (i.p.) injected in a volume of 0.1 ml/100g body weight.

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The cannabinoids were also injected i.p. in doses as follows: HU-210 – 0.4 mg/kg, AM251 – 1 mg/kg, JTC-801 – 1 mg/kg, AA-5-HT – 5 mg/kg. The doses and the time intervals of administration of the substances are in accordance with the data found in the literature [12–15].

In our experiments was used N/OFQ(1-13)NH₂, nociceptin fragment, exerting the full activity of nociceptin.

Rats were divided into experimental groups (6 animals each) as follows: (1) Controls – CG-treated; (2) Controls – treated with CG + DMSO; (3) treated with N/OFQ(1-13)NH₂ simultaneously with CG; (4) treated with [Orn⁹]N/OFQ(1-13)NH₂ 15 min before carrageenan; (5) treated with CB₁-receptors agonist HU-210 + CG; (6) treated with the blocker of CB₁-receptors AM251 + N/OFQ(1-13)NH₂; (7) treated with the blocker of CB₁-receptors AM251 + [Orn⁹]N/OFQ(1-13)NH₂; (8) treated with the blocker of NOP-receptors JTC-801 + N/OFQ(1-13)NH₂; (9) treated with the blocker of NOP-receptors JTC-801 + [Orn⁹]N/OFQ(1-13)NH₂; (10) treated with the blocker of TRPV1-receptors AA-5-HT + N/OFQ(1-13)NH₂; (11) treated with the blocker of TRPV1-receptors AA-5-HT + [Orn⁹]N/OFQ(1-13)NH₂.

The experiments were performed according to the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences, as well
as according to the European Convention for Protection of Experimental Animals (Protection of animals used for experimental purposes, Council Directive 86/609/EEc of November 1986).

The data were compared by unpaired Student-Fisher t-test, where $p < 0.05$ was considered statistically significant.

The chemicals: HU-210, AM251, JTC-801 and AA-5-HT, used in the present study, were purchased from Tocris and DMSO (dimethyl sulfoxide) and λ-carrageenan (CG) was from Sigma-Aldrich. N/OFQ(1-13)NH$_2$ and [Orn$^9$]N/OFQ(1-13)NH$_2$ were synthesized in the Department of Organic Chemistry, University of Chemical Technology and Metallurgy (Sofia, Bulgaria). The solutions of N/OFQ(1-13)NH$_2$ and [Orn$^9$]N/OFQ(1-13)NH$_2$ were freshly prepared in saline before each experiment. CG was prepared as a 1%-solution in 1% dimethylcellulose. The cannabinoid agents were dissolved in DMSO prior to the experiments.

**Results and discussion.** In our experiments, N/OFQ(1-13)NH$_2$ and [Orn$^9$]N/OFQ(1-13)NH$_2$ were used as agonists of the NOP-receptors [16].

The experiments confirmed our previous findings, that N/OFQ(1-13)NH$_2$ (applied simultaneously with CG) and [Orn$^9$]N/OFQ(1-13)NH$_2$ (applied 15 min before CG) in a dose of 20 μg/kg suppress the carrageenan-induced paw-oedema inflammation [17]. Our observation indicates the potential value of these pep-

![Bar chart](image.png)

**Fig. 2.** Effects of HU-210, [Orn$^9$]N/OFQ(1-13)NH$_2$ and their combination on carrageenan-induced acute inflammation in rat hind paw. Values represent the mean ± SEM of 6 animals. Statistically significant differences versus control with CG $^* p < 0.05$, and versus control with CG+DMSO $^# p < 0.05$, $^{##} p < 0.01$, $^{###} p < 0.001$

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tides for treatment of pain, oedema, cough, drug dependence, stress-induced anorexia, etc.

The activation of CB\textsubscript{1}-receptors by the potent agonist HU-210 significantly reduced the volume of the inflamed paw compared to the controls (group treated with CG + DMSO). The effect was observed in the entire period of the experiment and was much more pronounced than that of NOP-agonists applied alone (Figs 1, 2). However, the combined application of the two type receptor agonists (NOP and CB\textsubscript{1}) did not further increase the anti-inflammatory effect.

Injected after blockade of CB\textsubscript{1}-receptors, neither N/OFQ(1-13)NH\textsubscript{2}, nor [Orn\textsuperscript{9}]N/OFQ(1-13)NH\textsubscript{2} exerted their inhibitory effects on inflammation. Furthermore, from the 2nd h to the end of the observation (4 h), the measured paw volume was even elevated in the group treated with AM251 and N/OFQ(1-13) NH\textsubscript{2}, although the difference with the control values was not significant (Fig. 3). Obviously, the blockade of the CB\textsubscript{1}-receptors abolished the anti-inflammatory action of the tested peptides. Based on the experimental data, we supposed that activated CB\textsubscript{1}-receptors are necessary for nociceptin agonists to exhibit their anti-inflammatory effects. It might also be assumed that N/OFQ(1-13)NH\textsubscript{2} itself activates CB\textsubscript{1}-receptors. The insignificant increase in inflamed-paw volume observed in this group is most probably a consequence of the well-known additional effects of nociceptin, including release of histamine or other pro-inflammatory mediators.

Fig. 3. Effects of N/OFQ(1-13)NH\textsubscript{2} after AM251 treatment on carrageenan-induced acute inflammation in rat hind paw. Values represent the mean ± SEM of 6 animals. Statistically significant differences versus control with CG *\(p < 0.05\), **\(p < 0.01\), ***\(p < 0.001\).
On the contrary, the effect of HU-210 on inflamed-paw volume remains unaffected by pre-treatment of the rats with the NOP-receptor blocker JTC-801 (data not shown). However, when the CB1-agonist was injected after vanilloid-receptor inhibitor AA-5-HT, it did not cause further decrease in the studied parameters (data not shown). This confirms that the effect of the CB1-cannabinoid agonist is mediated by these receptors [18–20]. On the other hand, the blockade of vanilloid-receptors, in general, did not affect the anti-inflammatory action of N/OFQ(1-13)NH2 and [Orn9]N/OFQ(1-13)NH2 – the paw volume in the groups treated/not treated with AA-5-HT was very similar (Fig. 4). In all experiments, nociceptin ligands were less effective than AA-5-HT alone.

Summarized, our results have shown that the anti-inflammatory action of cannabinoid CB1-agonists is not related to the functional state of nociceptin receptor, while unoccupied or activated CB1-receptors are required for nociceptin to express fully its anti-inflammatory action. Dependence between CB1- and NOP-receptors has also been found in the production of hypothermia in conscious rats [15] where the NOP-receptors mediate the cannabinoid-induced effect.

To elucidate completely the mechanism of interaction between cannabinoid and nociceptin systems on acute peripheral inflammation, future studies with CB2- and NOP-agonists are needed.

Fig. 4. Effects of [Orn9]N/OFQ(1-13)NH2 after AA-5-HT treatment on carrageenan-induced acute inflammation in rat hind paw. Values represent the mean ± SEM of 6 animals. Statistically significant differences versus control with CG *p < 0.05, **p < 0.01.

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To elucidate completely the mechanism of interaction between cannabinoid and nociceptin systems on acute peripheral inflammation, future studies with CB2- and NOP-agonists are needed.
The concept suggested by the results of this work can be expected to provide new avenues for the development of drugs for combating diseases accompanied by inflammation, such as asthma, arthritis, gastritis, myocarditis, etc.

REFERENCES


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