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Research Article

Involvement of the opioidergic and nociceptinergic systems in the analgesic effects of novel nociceptin analogues after acute and chronic immobilization stress

Ivelina Himcheva[1], Galya Tz. Stavreva[1], Emilia Naydenova[2], Adriana Bocheva[1]

1 *Medical University of Pleven, Faculty of Medicine, Pleven, Bulgaria*
2 *University of Chemical Technology and Metallurgy, Sofia, Bulgaria*

Corresponding author: Ivelina Himcheva (himcheva7@abv.bg)

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Abstract

Stress is known to exert an influence on neuroendocrine, autonomic, hormonal functioning. Various stress models have been reported to induce analgesia. This is a phenomenon, referred to as stress-induced analgesia (SIA). Nociceptin/Orphanin FQ(N/OFQ) is a heptadecapeptide that has been found to play a direct role on pain perception.

This study aimed to investigate the effects of novel nociceptin analogues on nociception after acute and chronic immobilization stress (CIS) and the involvement of the opioid and nociceptinergic systems in analgesic effects. Analgesic effects were examined by paw-pressure (PP) and hot-plate (HP) tests.

Our data showed that acute immobilization stress induced hypoalgesia. The analgesic effect was more pronounced in pain caused by a mechanical stimulus than by a thermal one. CIS attenuated the hyperalgesic effect of naloxone and JTC-801 for mechanical and thermal stimulation. The effects of the opioid system are more pronounced in acute immobilization stress, while the nociceptin mechanisms predominate after chronic stress.

Keywords

JTC-801, immobilization stress, naloxone, nociceptin analogues

Introduction

Stress causes functional and structural changes in the body as a result of the interaction between the central nervous system (**CNS**), endocrine and immune systems (Pacak and Palkovits 2001). Opioid peptides are released during stress, leading to antinociceptive effects. This is a phenomenon, referred to as stress-induced analgesia (SIA). SIA is observed in many species of animals and can be caused by various stressors - immobilization, low, high temperature, social stressors (Miczek et al. 1982; Teskey et al. 1984; Lester and Fanselow 1985; Kavaliers and Innes 1987; Kavaliers 1990; Kavaliers and Colwell 1991). SIA is categorized as: opioid and non-opioid. The non-opioid component includes a number of neurotransmitter systems: adrenergic (Micutkova et al. 2001), serotonergic, endocannabinoid

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(Finn 2010), nociceptinergic (Gavioli and Calo' 2013; Witkin et al. 2014; Gavioli et al. 2019), which alter pain perception and modulation of behavioral responses after stress (Pacak and Palkovits 2001; Butler and Finn 2009).

One of the mechanisms known to play a part in the response of an organism to stress is activation of the endogenous opioid system. The wide distribution of opioidergic neurons and opioid receptors in the CNS and peripheral nervous system determines the participation of the opioid system in the control of analgesia, neuroendocrine secretion, locomotor activity, learning and memory, addiction and tolerance (Cesselin 1995; Millan et al. 1996).

Molecules that are classified as anti-opioids are synthesized and released in the CNS. Nociceptin/orphanin FQ (**N/OFQ**), neuropeptide FF (**NPFF**), cholecystokinin (**CCK**), melanocyte inhibiting factor (**MIF**)-related peptides and others have anti-opioid properties (Cesselin 1995). Anti-opioids have morphinomimetic, anti-opioid effects antagonized by naloxone (Kastin et al. 1979; Cesselin 1995).

Nociceptin/Orphanin FQ (N/OFQ) is derived from pro-nociceptin/orphanin FQ (Mogil and Pasternak 2001; Rizzi et al. 2002). The amino acid sequence of nociceptin is very similar to that of other opioid peptides, especially Dynorphin A, which is evidence of the close evolutionary link between precursors. The first amino acid at the N-terminal of nociceptin is Phe, while the other opioid peptides contain the following fragment: Tyr^1-Gly^2-Gly^3-Phe^4-Met^5/Leu^5. These peptides have different affinity for μ (MOR), δ (DOR), and κ (KOR) opioid receptors (Pasternak and Wood 1986; Besse et al. 1990; Reisine and Bell 1993; Kieffer 1995), while they have low affinity for the nociceptin opioid peptide (NOP) receptor (Mollereau et al. 1996; Mogil and Pasternak 2001).

It has been reported that the N/OFQ-NOP receptor system modulates several biological functions, including pain transmission, stress and anxiety, learning and memory, food intake (Mogil and Pasternak 2001; Rizzi et al. 2002). The activity of N/OFQ in pain modulation was different when it was administered in the brain versus the spinal cord, this also depended on the assay used (Toll et al. 2019). Many biological actions of N/OFQ are reported to alleviate behavioral and sensory responses to stress, such as fear responses (Jenck et al. 1997) and analgesia (Rizzi et al. 2001).

A growing body of evidence suggests that stress modulates endogenous N/OFQergic signaling. This includes: evidence regarding the distribution of the peptide N/OFQ and the NOP protein in brain regions important in stress (Witkin et al. 2014); the effects of stress in regulating N/OFQ release and NOP expression (Devine et al. 2001); the ability of the N/OFQ-NOP system to modulate the hypothalamic-pituitary-adrenal (HPA) axis; behavioral data obtained with selective NOP agonists and antagonists, as well with NOP knockout (NOP(-/-)) animals in different stress-related animal models.

The aim of this study was to investigate the analgesic effects of novel analogues of N/OFQ(1-13)NH_2, where Lys at position 9 and/or 13 was substituted by Orn on nociception after acute and chronic immobilization stress and the involvement of the opioid and nociceptinergic systems in these effects.

Materials and methods

Chemisrty

The protected amino acids and Fmoc-Rink Amide MBHA Resin were purchased from Iris Biotech (Germany). All other reagents and solvents were analytical or HPLC grade and were bought from Merck (Germany). The LC/MC spectra were recorded on a LTQ XL Orbitrap Discovery instrument, Thermo Corporation, USA. The optical rotation was measured on automatic standard polarimeter Polamat A, Carl Zeis, Jena. The conventional solid-phase peptide synthesis based on Fmoc (9-fluorenylmethoxycarbonyl) chemistry was employed to synthesize a series of new analogues of N/OFQ (1-13). Rink-amide MBHA resin and TBTU (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate) or DIC (N,N'-Diisopropylcarbodiimide) were used as solid-phase carrier and condensing reagent. Three-functional amino acids were embedded as N^{α}-Fmoc-Thr(tBu)-OH, N^{α}-Fmoc-Lys(Boc)-OH, N^{α}-Fmoc-Orn(Boc)-OH, N^{α}-Fmoc-Arg(Pbf)-OH. The coupling reactions were performed, using for amino acid/TBTU/HOBt/DIEA/resin a molar ratio 3/3/3/9/1 or amino acid/DIC/HOBt/resin a molar ratio 3/3/3/1. The Fmoc-group was deprotected by a 20% piperidine solution in N,N-Dimethylformamide. The coupling and deprotection reactions were checked by the Kaiser test. The cleavage of the synthesized peptide from the resin was done, using a mixture of 95% trifluoroacetic acid (TFA), 2.5% triisopropylsilane (TIS) and 2.5% water. The peptide was obtained as a filtrate in TFA and precipitated with cold dry ether. The precipitate was filtered, dissolved in water and lyophilized to obtain the crude peptide. The peptide purity was monitored on a RP-HPLC XTera C18 3.5 μm (125×2.1 mm) (Waters Co.) column, flow 200 μl/min, using a linear binary gradient of phase B from 10% to 90% for 15 min (phase A: 0.1% HCOOH/H_2O; phase B: 0.1% HCOOH/AcCN). The compounds were checked by electrospray ionization mass spectrometry and the optical rotation was measured in water.

Animals

The experiments were carried out on 144 male Wistar rats (180–200 g) kept under normal conditions at ambient room temperature (22±2 °C), maintained under a 12 h/12 h light/dark regime, and supplied with standard chow and water ad libitum. The animals were divided into 24 groups, each one consisted of 6 animals. All experiments were performed by the requirements of the Bulgarian Food Safety Agency for work with animals with a registration license № 239.

Acute model of immobilization stress

The animals are placed for 1 hour in special transparent plastic cylinders with breathing holes, but limiting their movements to a minimum.

Chronic model of immobilization stress

The animals are placed for 3 hours daily for 4 days in special transparent plastic cylinders with breathing holes, limiting their movements to a minimum.

Nociceptive tests

Paw-pressure test

The evaluation of antinociceptive effects was carried out using the paw-pressure (PP) test (Randall and Selitto 1957). The changes in the mechanical nociceptive thresholds of the rats were measured by an analgesimeter (Ugo Basile). The pressure was applied to the rat hind-paw and the pressure (g) required for eliciting a nociceptive response, such as a squeak or struggle, was taken as the mechanical nociceptive threshold. A cut-off value of 500 g was observed to prevent damage of the paw.

Hot-plate (HP) test

Thermal nociceptive stimulus is applied to the paws of freely mobile animals. The latency of response to pain was measured from the moment the animal was placed on the metal plate (heated to 55 ± 0.5 °C) till the first signs of pain (paw licking, jumping). A cut-off time of 30 sec. was observed in order to avoid injury of the animals. Time is reported in seconds.

Drugs and treatment

Nociceptin analogues were synthesized in the laboratory of Prof. Ph.D. E. Naydenova in the University of Chemical Technology and Metallurgy – Sofia. All novel analogues of N/OFQ were injected at a dose of 10 μg/kg. All drugs were obtained from Sigma. Naloxone (Nal, non-selective opioid receptor antagonist, 1 mg/kg), was applied immediately after the end of stress and 20 min before each peptide. JTC-801 (NOP receptor antagonist, 0.5 mg/kg), was administered immediately after the end of stress and 10 min before each peptide. All drugs were dissolved in saline (0.9% NaCl) solution and were injected intraperitoneally (i.p.). The control group was not submitted to stress procedure and was injected with saline 0.1 ml/kg, i.p.

The experimental studies were performed following several protocols:

1) In the first place the impact of acute one hour of immobilization stress (1hIS) and chronic immobilization stress (CIS) on nociception was estimated and the effects of N/OFQ and novel nociceptin analogues administration immediately after the end of 1hIS and CIS;
2) The participation of the opioidergic system in the analgesic effects of the novel nociceptin analogues was confirmed by Naloxone pretreatment applied immediately after stress (1hIS/CIS) before novel nociceptin analogues;
3) The participation of the nociceptinergic system in the analgesic effects of the novel nociceptin analogues was confirmed by JTC-801 pretreatment applied immediately after stress (1hIS/CIS) before novel nociceptin analogues.

Data analysis

The results were presented as mean values±S.E.M. and were tested by one-way ANOVA, followed by Fisher's least significant difference procedure as a post-hoc test. The differences between the groups were considered statistically significant at $p \leq 0.05$. Analyses were performed using STATGRAPHICS Centurion XV statistical software.

Results and discussion

In order to study and establish the influence of the amino acids Lys9 and Lys13, we synthesized by Solid Phase Peptide Synthesis, Fmoc-strategy, the following new fragment analogs of N/OFQ(1-13)NH_2:

[Orn^9]N/OFQ(1-13)NH_2:H-Phe^1-Gly^2-Gly^3-Phe^4-Thr^5-Gly^6-Ala^7-Arg^8-Orn^9-Ser^{10}-Ala^{11}-Arg^{12}-Lys^{13}-NH_2

[Orn^9,Orn^{13}]N/OFQ(1-13)NH_2:H-Phe^1-Gly^2-Gly^3-Phe^4-Thr^5-Gly^6-Ala^7-Arg^8-Orn^9-Ser^{10}-Ala^{11}-Arg^{12}-Orn^{13}-NH_2

The compounds were tested on nociception after acute and chronic immobilization stress (CIS) and the involvement of the opioidergic and nociceptinergic systems in these effects.

Effects of novel nociceptin analogues after acute immobilization stress

In the first experiment one hour of immobilization stress (1hIS) increased the pain threshold and hot-plate latency of experimental animals compared to the controls. The analgesic effect was more pronounced in pain caused by a mechanical stimulus (Fig. 1) than by a thermal one (Fig. 2). Our data showed that on the 10th, 20th and 30th min of the experiment N/OFQ(1-13)NH_2, [Orn^9]N/OFQ(1-13)NH_2 and [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 decreased significantly ($p < 0.05$) pain threshold compared to a group that underwent acute stress only.

Pronounced stress-induced analgesia was observed at the 10th min after the acute immobilization stress. In the PP test, the pain threshold increased almost twice (94.5%) at the 10th min, and the antinociceptive effect continued throughout the study period. In the HP test involving

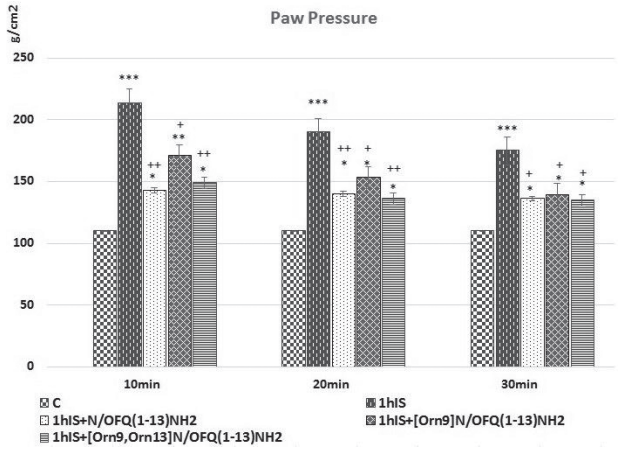

Figure 1. Effects of nociceptin N/OFQ(1-13)NH_2 and novel nociceptin analogues [Orn^9]N/OFQ(1-13)NH_2, [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 (all at a dose 10 μg/kg, i.p) on nociception measured by Paw-Pressure (PP) test in male Wistar rats after 1 hour of immobilization stress (1hIS). Mean values ± S.E.M. are presented (*p < 0.05; **p < 0.01 vs. control; $^{+}$p < 0.05; $^{++}$p < 0.01 vs. 1hIS).

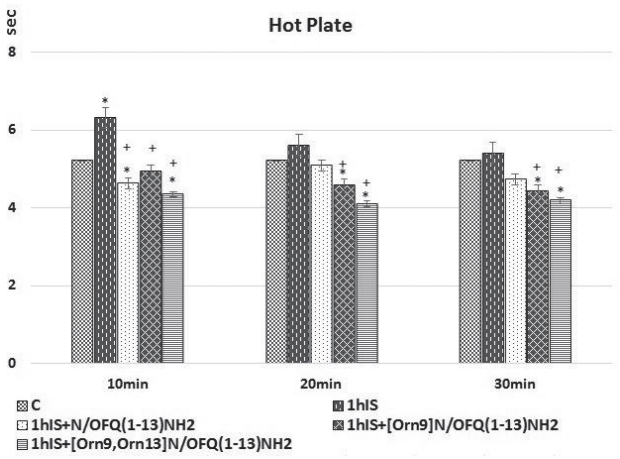

Figure 2. Effects of nociceptin N/OFQ(1-13)NH_2 and novel analogues [Orn^9]N/OFQ(1-13)NH_2, [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 (all at a dose 10 μg/kg, i.p) on nociception measured by Hot-Plate (HP) test in male Wistar rats after 1 hour of immobilization stress (1hIS). Mean values ± S.E.M. are presented (*p < 0.05 vs. control; $^{+}$p < 0.05 vs. 1hIS).

thermoreceptors, the effect was statistically significant only at the 10th min (latency increased by 21%).

Literature data showed that immobilization stress causes an increase in antinociception in tail-flick (Aloisi et al. 1998), hot-plate (Amir and Amit 1978), and formalin tests (Appelbaum and Holtzman 1985). The evidence that N/OFQ decreased tail-flick and hotplate latency, when centrally administered, was reported with the discovery of this peptide (Meunier et al. 1995).

Naloxone was used in the second experiment to investigate the involvement of the opioidergic system in the analgesic effects of the analogues. The obtained results showed that naloxone applied immediately after 1hIS decreased significantly (p < 0.001) pain threshold. Naloxone induced hyperalgesia, more pronounced for mechanical pain. Co-administration of nociception and analogues with naloxone after ending of stress decreased significantly (p < 0.05) pain threshold and hot-plate latency for the whole period of the study compared to a group that underwent acute stress only (Figs 3, 4).

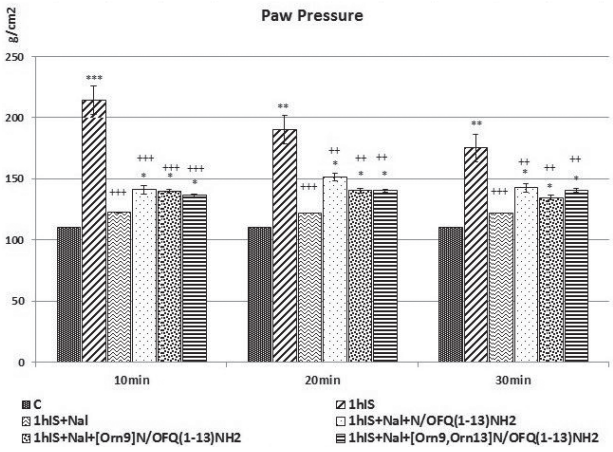

Figure 3. Effects of Naloxone (Nal, 1 mg/kg, i.p) when co-administered with N/OFQ(1-13)NH_2 and novel analogues [Orn^9]N/OFQ(1-13)NH_2, [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 (all at a dose 10 μg/kg, i.p) on the pain threshold measured by PP test in male Wistar rats after 1hIS. Mean values ± S.E.M. are presented (*p < 0.05 vs. control; $^{++}$p < 0.01; $^{+++}$p < 0.001 vs. 1hIS).

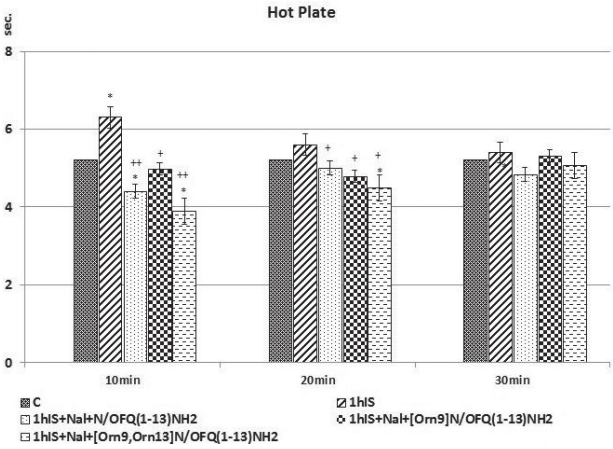

Figure 4. Effects of Naloxone (Nal, 1 mg/kg, i.p) when co-administered with N/OFQ(1-13)NH_2 and novel analogues [Orn^9]N/OFQ(1-13)NH_2, [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 (all at a dose 10 μg/kg, i.p) on nociception measured by HP test in male Wistar rats after 1hIS. Mean values ± S.E.M. are presented (*p < 0.05 vs. control; $^{+}$p < 0.05; $^{++}$p < 0.01 vs. 1hIS).

Literature and previous data of ours showed that the antinociception induced by i.c.v. L-Orn was abolished by naloxone and naltrindole and suggested the involvement of opioid receptors (Kawabata et al. 1996; Dzambazova et al. 2008). Considering these findings it was not surprising that naloxone inhibited the analgesic effects of newly synthesized N/OFQ(1-13)NH_2 analogues which suggested the involvement of opioidergic system.

In the third experiment, the effect of nociceptin and analogues on the nociceptin neurotransmitter system after 1hIS

was studied. JTC-801 (NOP receptor antagonist) was administered at a dose of 0.5 mg/kg, i.p. immediately after the end of stress and 10 min before administration of the peptides. Nociceptin and analogues [Orn^9]N/OFQ(1-13)NH$_2$, [Orn^9,Orn^{13}]N/OFQ(1-13)NH$_2$ administered with JTC-801 after immobilization stress decreased the pain threshold significantly ($p < 0.05$) on 10th, 20th, and 30th min and hot-plate latency ($p < 0.05$) compared to a group that underwent acute stress only (Figs 5, 6). Our results showed that [Orn^9,Orn^{13}]N/OFQ(1-13)NH$_2$ decreased the pain threshold more pronounced compared to the [Orn^9]N/OFQ(1-13)NH$_2$. The obtained results suggest the participation of nociceptinergic system in the analgesic effects of nociceptin and analogues.

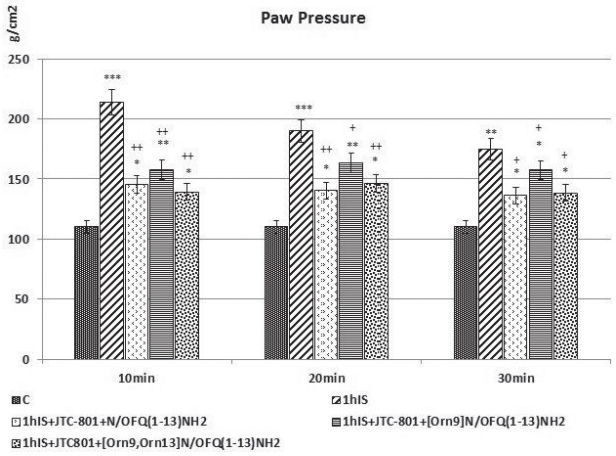

Figure 5. Effects of JTC-801 (0.5 mg/kg, i.p) co-administration with N/OFQ(1-13)NH$_2$ and novel analogues [Orn^9]N/OFQ(1-13)NH$_2$, [Orn^9,Orn^{13}]N/OFQ(1-13)NH$_2$ (all at a dose 10 μg/kg, i.p) on nociception measured by PP test in male Wistar rats after 1hIS. Mean values ± S.E.M. are presented (*$p < 0.05$; **$p < 0.01$ vs. control; $^{+}p < 0.05$; $^{++}p < 0.01$ vs. 1hIS).

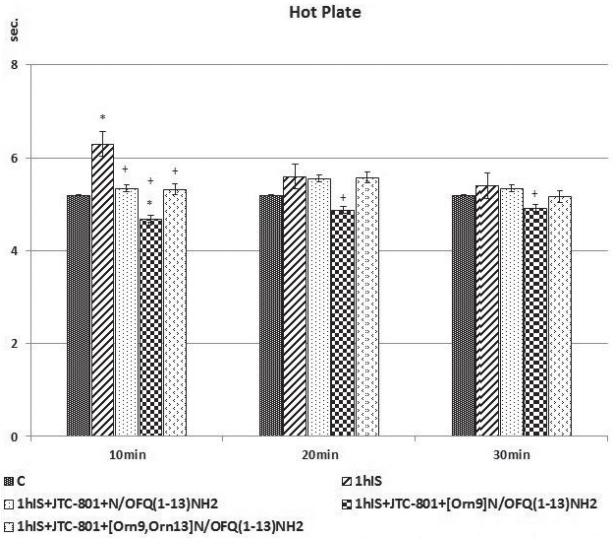

Figure 6. Effects of JTC-801 (0.5 mg/kg, i.p) co-administration with N/OFQ(1-13)NH$_2$ and novel analogues [Orn^9]N/OFQ(1-13)NH$_2$, [Orn^9,Orn^{13}]N/OFQ(1-13)NH$_2$ (all at a dose 10 μg/kg, i.p) on nociception measured by HP test in male Wistar rats after 1hIS. Mean values ± S.E.M. are presented (*$p < 0.05$ vs. control; $^{+}p < 0.05$ vs. 1hIS).

Effects of novel nociceptin analogues after chronic immobilization stress

To evaluate the effects of opioid and nociceptin neurotransmissions after chronic immobilization stress (CIS), we conducted a series of experiments with a design similar to that of acute immobilization stress. The animals were immobilized for 3 hours daily for 4 days. Naloxone and JTC-801 were administered immediately after the end of stress.

Our results showed that CIS caused a slight increase in pain threshold and hot-plate latency, which were not statistically significant versus the controls. Nociceptin and analogues [Orn^9]N/OFQ(1-13)NH$_2$, [Orn^9,Orn^{13}]N/OFQ(1-13)NH$_2$ administered after CIS decreased the pain threshold and hot-plate latency significantly ($p < 0.05$) compared to a group that underwent chronic stress only. The newly synthesized analogues of N/OFQ(1-13)NH$_2$, in which the Lys at the 9th and 13th positions substituted with Orn suppressed the pain threshold more strongly than that of [Orn^9]N/OFQ(1-13)NH$_2$ after CIS (Figs 7, 8).

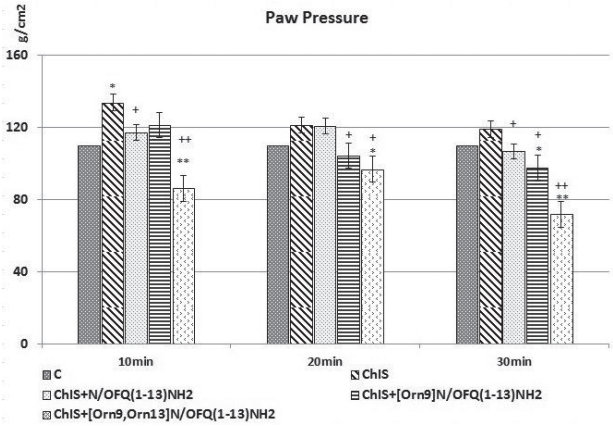

Figure 7. Effects of nociceptin N/OFQ(1-13)NH$_2$ and novel nociceptin analogues [Orn^9]N/OFQ(1-13)NH$_2$, [Orn^9,Orn^{13}]N/OFQ(1-13)NH$_2$ (all at a dose 10 μg/kg, i.p) on nociception measured by PP test in male Wistar rats after chronic immobilization stress (CIS). Mean values ± S.E.M. are presented (*$p < 0.05$; **$p < 0.01$ vs. control; $^{+}p < 0.05$; $^{++}p < 0.01$ vs. CIS).

The administration of naloxone led to significantly reduced values of the pain threshold and hot-plate latency after the end of chronic immobilization ($p < 0.05$). The effect of naloxone was more significant in the HP test (latency decreased by 72%) compared to the PP test (pain threshold decreased by 36%). Simultaneously application of naloxone with nociceptin and analogues reduced the mechanical threshold and thermal pain significantly ($p < 0.05$) compared to a group that underwent chronic stress only (Figs 9, 10).

Chronic immobilization stress did not evoke hypoalgesia, but attenuated the hyperalgesic effect of naloxone for mechanical and thermal stimulation.

Application of JTC-801 after the end of chronic immobilization significantly reduced ($p < 0.05$) the pain threshold and hot-plate latency compared to a group that

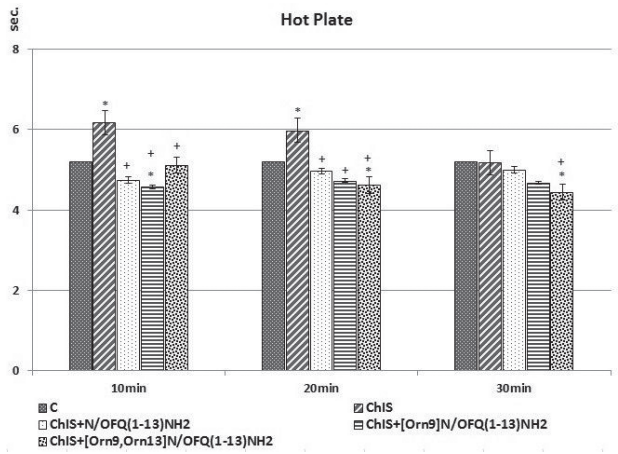

Figure 8. Effects of nociceptin N/OFQ(1-13)NH_2 and novel nociceptin analogues [Orn^9]N/OFQ(1-13)NH_2, [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 (all at a dose 10 μg/kg, i.p) on nociception measured by HP test in male Wistar rats after CIS. Mean values ± S.E.M. are presented (*p < 0.05 vs. control; $^{+}$p < 0.05 vs. CIS).

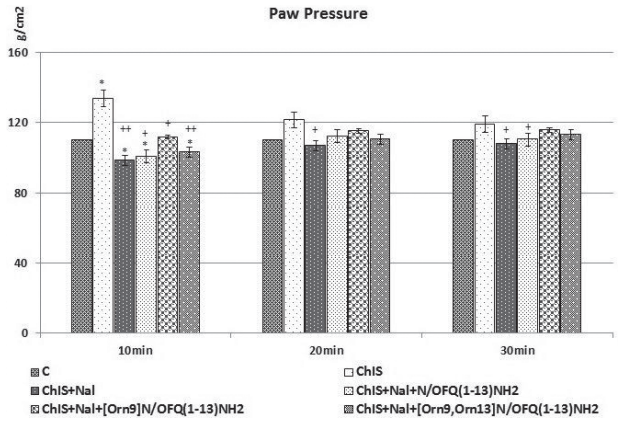

Figure 9. Effects of Naloxone (Nal, 1 mg/kg, i.p) co-administration with N/OFQ(1-13)NH_2 and novel analogues [Orn^9]N/OFQ(1-13)NH_2, [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 (all at a dose 10 μg/kg, i.p) on the pain threshold (PP) test in male Wistar rats after CIS. Mean values ± S.E.M. are presented (*p < 0.05 vs. control; $^{+}$p < 0.05 vs. CIS).

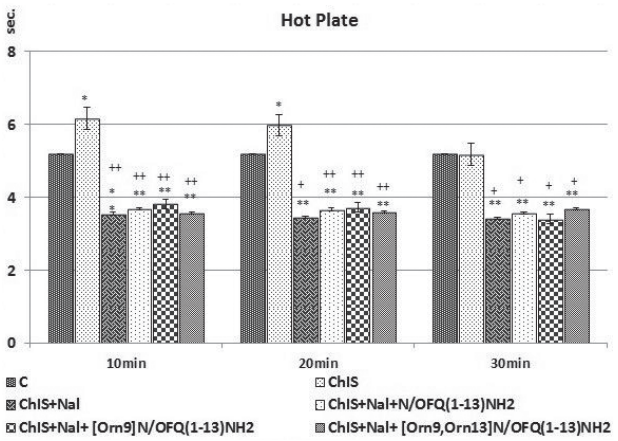

Figure 10. Effects of Naloxone (Nal, 1 mg/kg, i.p) co-administration with N/OFQ(1-13)NH_2 and novel analogues [Orn^9]N/OFQ(1-13)NH_2, [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 (all at a dose 10 μg/kg, i.p) on nociception measured by HP test in male Wistar rats after CIS. Mean values ± S.E.M. are presented (*p < 0.05; **p < 0.01 vs. control; $^{+}$p < 0.05; $^{++}$p < 0.01 vs. CIS).

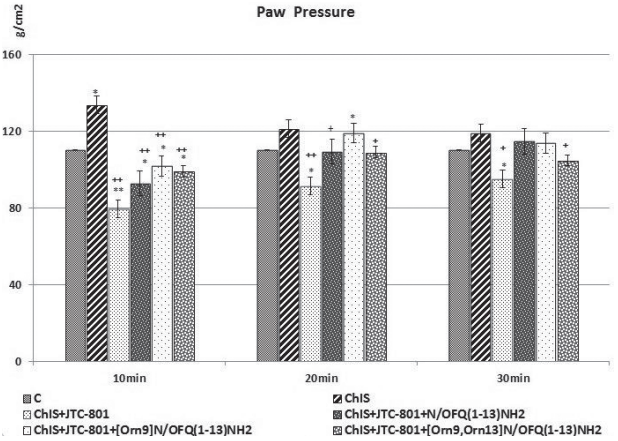

Figure 11. Effects of JTC-801 (0.5 mg/kg, i.p) co-administration with N/OFQ(1-13)NH_2 and novel analogues [Orn^9]N/OFQ(1-13)NH_2, [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 (all at a dose 10 μg/kg, i.p) on the pain threshold (PP) test in male Wistar rats after CIS. Mean values ± S.E.M. are presented (*p < 0.05; **p < 0.01 vs. control; $^{+}$p < 0.05; $^{++}$p < 0.01 vs. CIS).

underwent chronic stress only. Nociceptin and analogues [Orn^9]N/OFQ(1-13)NH_2, [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 administered with JTC-801 after CIS decreased the pain threshold and hot-plate latency significantly (p < 0.05) on 10^{th}, 20^{th} and 30^{th} min compared to a group with CIS only. The obtained results suggest the participation of nociceptinergic system in the analgesic effects of nociceptin and analogues after CIS (Figs 11, 12).

The obtained results give us reason to assume that chronic immobilization stress induces mild hypoalgesic effects, in which the modulation of pain perception is mediated primarily by the nociceptin neurotransmitter system.

Stress-induced analgesia is a key component of the "fight-or-flight" response. Immobilization is very often used as a model of stress-induced analgesia (Stevens et al. 1995; Goyal and Anil 2007). The data been shown that in addition to the nature of the stressor, the duration of action of the stimulus is extremely important for activating the opioid and non-opioid components that provide pain inhibition (Parikh et al. 2011). Literature data showed that opioid and non-opioid components are equally presented in immobilization stress (Pacak and Palkovits 2001; Finn 2010). It is also known that acute and chronic stress induce biochemical changes affecting both pain threshold and behaviour (Pacak and Palkovits 2001; Butler and Finn 2009). A number of mediators and neurotransmitter systems are involved in SIA – opioidergic, nociceptinergic (Witkin et al. 2014; Gavioli et al. 2019), adrenergic (Micutkova et al. 2001), endocannabinoid (Finn 2010) and others. The cross-talk between nociceptin and opioid systems, supported by anatomical, biochemical and molecular data is revealed (Gavioli et al. 2021).

The results suggest that acute and chronic immobilization stress induced hypoalgesia is mediated by opioid

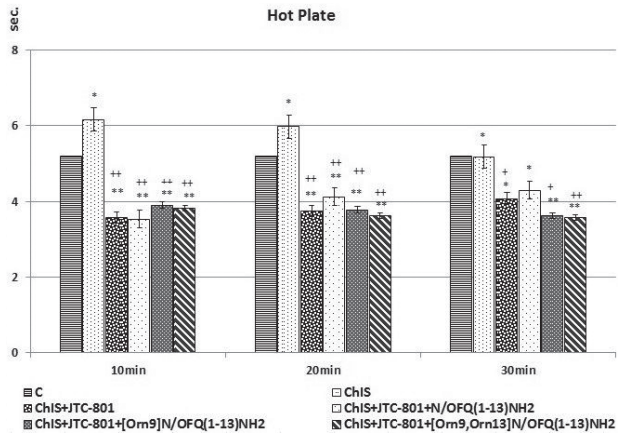

Figure 12. Effects of JTC-801 (0.5 mg/kg, i.p) co-administration with N/OFQ(1-13)NH_2 and novel analogues [Orn^9]N/OFQ(1-13)NH_2, [Orn^9,Orn^{13}]N/OFQ(1-13)NH_2 (all at a dose 10 μg/kg, i.p) on nociception measured by HP test in male Wistar rats after CIS. Mean values ± S.E.M. are presented (*p < 0.05; **p < 0.01 vs. control; $^{+}$p < 0.05; $^{++}$p < 0.01 vs. CIS).

receptors and nociceptin neurotransmission; mechanical pain effect is stronger than thermal one.

Our study demonstrated that substitution of Orn at position 9 and 13 in molecule of nociceptin decreased significantly the pain threshold of newly synthesized analogues after acute and chronic immobilization stress.

The newly synthesized analogues of N/OFQ(1-13)NH_2, in which the Lys at the 9th and 13th positions substituted with L-ornithine suppresses the pain threshold more strongly than that of [Orn^9]N/OFQ(1-13)NH_2 after acute and chronic immobilization stress.

Conclusion

For the first time, original results were obtained for the relationships between N/OFQ analogues and the opioid - and nociceptin neurotransmitter systems after acute and chronic immobilization stress. The data suggests that analgesic effects of N/OFQ analogues are influenced by non-selective inhibitor of opioid receptors and inhibitor of NOP receptor after acute and chronic immobilization stress.

In conclusion, the effects of the opioid and nociceptin systems are more pronounced in acute immobilization stress, while the nociceptin mechanisms predominate probably after chronic stress.

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