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ANALGESIC EFFECTS OF SOME NEWLY SYNTHESIZED NOCICEPTIN ANALOGUES IN RATS

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Summary. Since its discovery in 1995, nociceptin (N/OFQ) and its N-terminal tridecapeptide sequence (N/OFQ (1-13)) were objects of intense investigation in order to ascertain their innumerable biological properties. Later, after chemical substitution of individual amino acids, the effects on nociception of the newly synthesized analogues were investigated. The aim of the present study was to investigate whether newly synthesized analogues of N/OFQ (1-13), where Lys at position 9 and/or 13 was substituted by ornitine (Orn), diaminobutanoic acid (Dab) or diaminopropanoic acid (Dap) had analgesic effects, and the involvement of the opioidergic system in these effects. The experiments were carried out on male Wistar rats. The changes in the mechanical nociceptive pain threshold of the rats were measured by paw pressure test. Nociceptin (1-13) and the newly synthesized analogues were administered intraperitoneally (i.p.). Our data showed that nociceptin (1-13), [Orn⁹]N/OFQ(1-13)NH₂, [Orn¹³]N/OFQ(1-13)NH₂, [Orn^{9,13}]N/OFQ(1-13)NH₂ and [Dab⁹]NO/FQ (1-13) exerted a naloxone-reversible analgesic effect in which the opioidergic system was involved.

Key words: nociceptin analogues, analgesia, opioidergic system

АНАЛГЕТИЧНИ ЕФЕКТИ НА НЯКОИ НОВОСИНТЕЗИРАНИ АНАЛОЗИ НА НО-ЦИЦЕПТИН ПРИ ПЛЪХОВЕ

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Резюме. От откриването на ноцицептина през 1995 г. и неговия N-терминален тридекапептид – N/OFQ (1-13), се провеждат интензивно изследвания с цел установяване на някои от техните биологични ефекти. По-късно чрез химично субституиране на отделни аминокиселини в молекулата бяха изучени ефектите на новите аналози върху болковата перцепция. Целта на настоящото изследване бе изучаване аналгетичните ефекти на новосинтезирани аналози на N/OFQ (1-13), при които лизинът (Lys) на 9-а и 13-а позиция е субституиран с орнитин (Orn), диаминобутанова (Dab) или диаминопропанова (Dap) киселина, както и дали участва опиоидергичната система в тези ефекти. Използвани бяха мъжки плъхове порода Wistar (180-200 g). Промените в прага на механичната ноцицепция бяха измерени посредством paw pressure test. Ноцицептинът (1-13) и новосинтезираните аналози бяха въведени интраперитонеално. При нашите изследвания бе констатирано, че N/OFQ (1-13), [Orn⁹]N/OFQ(1-13)NH₂, [Orn¹³]N/OFQ(1-13)NH₂, [Orn^{9,13}]N/OFQ(1-13)NH₂ and [Dab⁹]NO/FQ (1-13) притежават налксонзависим аналгетичен ефект, което показва участието на опиоидната система.

Ключови думи: ноцицептинови аналози, аналгезия, опиоидергична система

Introduction

In 1995, nociceptin (NO/FQ), also named orphanin, was individuated to be the endogenous ligand of the opioid receptor-like 1 (ORL₁), previously referred to as “orphan” receptor [1]. The novel heptadecapeptide, whose sequence resembles to same extent that of the opioid peptide dynorphin A, was shown to modulate several biological functions: cardiovascular, immune competence, respiratory, gastrointestinal, urogenital, CNS's – nociception,

stress, anxiety, memory, learning, locomotion, motivation, feeding, reflecting the vast distribution (central and peripheral) of its specific receptor [3].

The N-terminal tridecapeptide sequence of nociceptin molecule, NO/FQ (1-13), was discovered to fulfill all the biological activities of NO/FQ, with arginine and lysine being crucial for receptor binding [5, 7]. Since their discovery, NO/FQ and NO/FQ (1-13) were largely investigated in order to determine the functional importance of their amino acid

sequence. Many analogues were also examined in the search of variations of their functions compared to the "original" molecule [7].

Since lysine (Lys) at the 9th and 13th positions has been estimated to be important for the receptor binding [4, 8, 9], we set out in search for how did structural analogues of Lys, Ornithine (Orn), diaminobutanoic acid (Dab) or diaminopropanoic acid (Dap) modify the analgesic activity of newly synthesized molecules.

The aim of the present study was to investigate the effects of newly synthesized analogues of N/OFQ (1-13), where Lys at position 9 and/or 13 was substituted by Orn, Dab or Dap, and the involvement of the opioidergic system in these effects.

Materials and methods

Animals. The experiments were carried out on male Wistar rats (180-200 g) kept under normal conditions at ambient room temperature (22°C). Ethical guidelines of the International Association for the Study of Pain in conscious animals were followed [10].

Assessment of algisia consisted of measurement of the threshold stimulus for reaction (escape or paw withdrawal) using a weight (maximum limit of 500 g) applied to the pads of hind paws by an experimenter using an Ugo Basile apparatus; this is essentially the method of Randall & Selitto, 1957 [6].

Drugs: naloxone (Nal) and nociceptin N/OFQ (1-13) were obtained from Sigma (Germany). Nociceptin analogues were synthesized in the laboratory of Assoc. Prof. Ph.D. E. Naydenova in the University of Chemical Technology and Metallurgy – Sofia. All drugs were dissolved in saline and injected intraperitoneally. Nal (1 mg/kg, i.p.) was applied 20 min before each peptide (10 µg/kg, i.p.). The control group received saline (1ml/kg, i.p.).

Statistics. All results were expressed as mean ± SEM. One-way analysis of variance was used to verify the statistical significance at $p < 0.05$ between treated and control groups.

Results and discussion

Our experiment showed that N/OFQ (1-13), all its investigated Orn-analogues and the Dab⁹-analogue exerted a significant time-dependent analgesic effect, while [Dab^{9,13}]N/OFQ(1-13)NH₂, [Dap⁹]N/OFQ(1-13)NH₂ and [Dap^{9,13}]N/OFQ(1-13)NH₂ had no analgesic effect compared to the control group (Fig. 1).

On the 10th min of the experiment only [Orn¹³]N/OFQ(1-13)NH₂ showed statistically significant ($p < 0.05$) analgesic effect compared to N/OFQ (1-13). On the 20th min its analgesic effect decreased (Fig. 1).

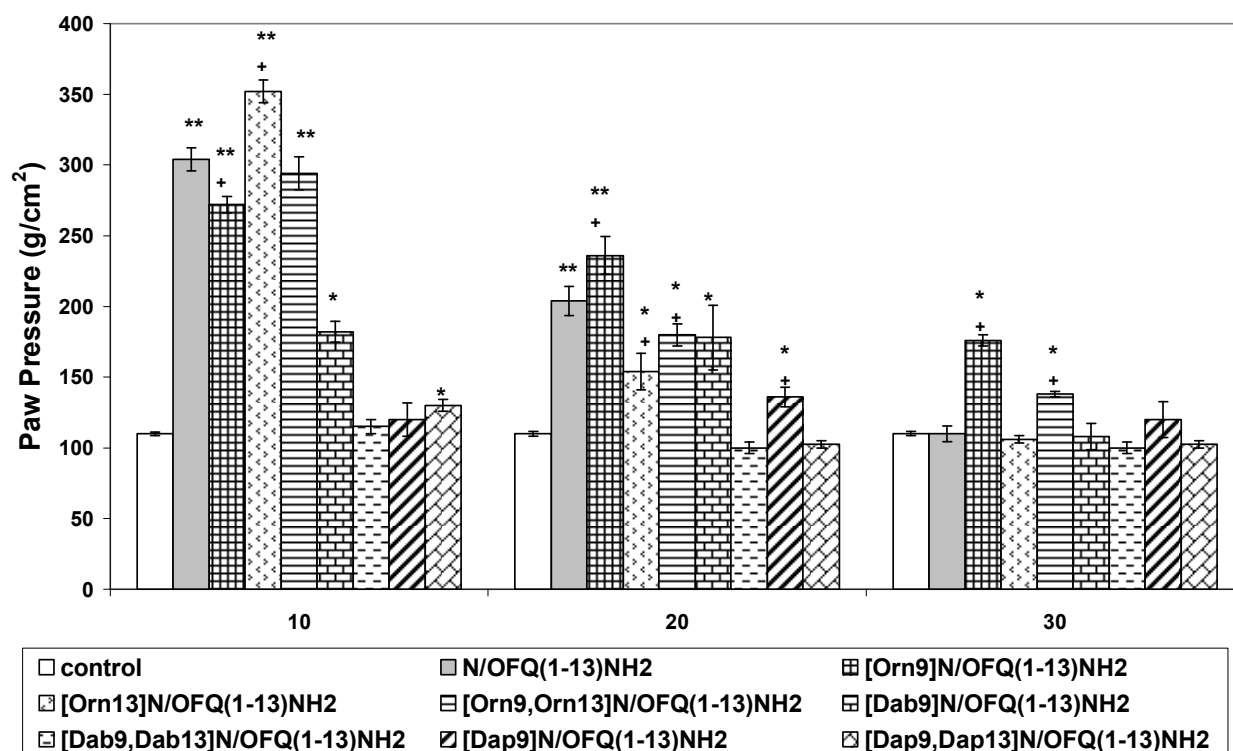


Fig. 1. Effects of i.p. administration of nociceptin and analogues (all at a dose of 10 µg/kg) estimated by paw pressure test. Data are presented as mean ± S.E.M.; * $P < 0.01$, ** $P < 0.01$ vs. control; + $P < 0.01$, ++ $P < 0.01$ vs. nociceptin

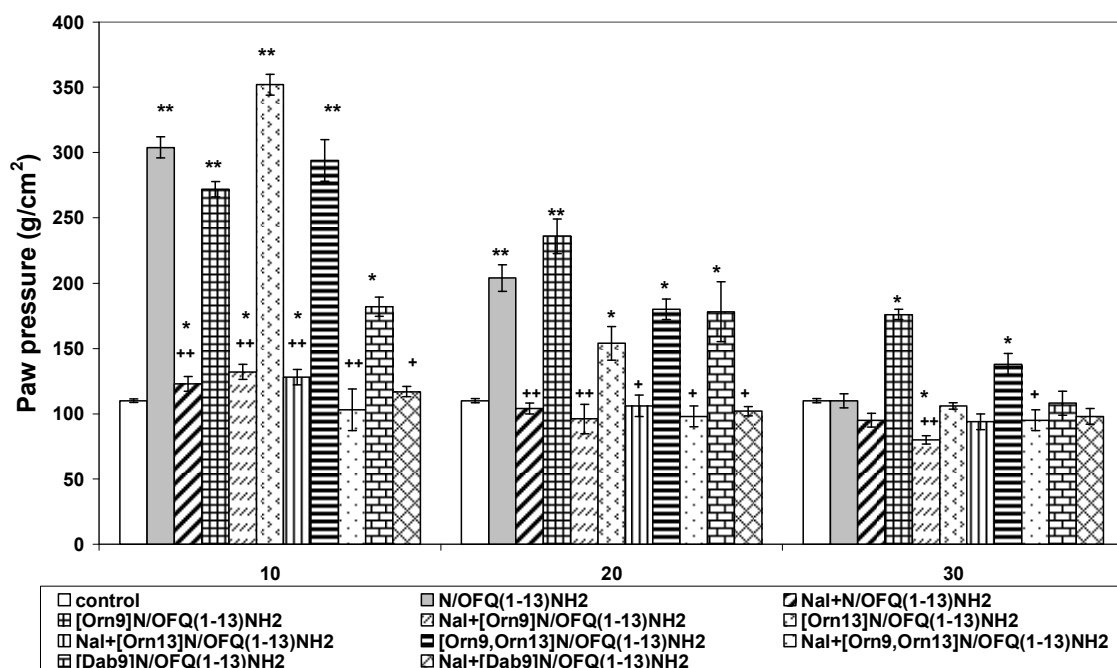


Fig. 2. Effects of i.p. administration of nociceptin and analogues (all at a dose of 10 µg/kg) after Nal administration estimated by paw pressure test. Data are presented as mean ± S.E.M.; *P < 0.01, **P < 0.01 vs. peptides respectively

On the 20th min of the experiment, the analgesic effect of [Orn⁹]N/OFQ(1-13)NH₂ was statistically significant ($p < 0.01$) compared to N/OFQ (1-13) (Fig. 1).

The analgesic effects of the other newly synthesized Orn-analogues were statistically significantly decreased ($p < 0.01$). On the 10th and the 20th min the analgesic effect of [Dap⁹]N/OFQ(1-13)NH₂ remained the same (Fig. 1).

On the 30th min, only [Orn⁹]N/OFQ(1-13)NH₂ ($p < 0.05$) and [Orn^{9,13}]N/OFQ(1-13)NH₂ ($p < 0.05$) had statistically significant analgesic effects compared to control group and N/OFQ (1-13) (Fig. 1). Our experiments confirmed that Lys at the 9th and 13th positions was important for the receptor binding and its structural analogues were able to modify analgesic activity of newly synthesized molecules [5]. Since the i.c.v. administration of neuroactive amino acid L-ornithine elicited significant antinociception in the mechanical and thermal nociception tests in intact mice [2], we suggest that this is one of the reasons that modification of the parent molecule NO/FQ (1-13) at position 13 with Orn leads to compound with more pronounced analgesic effect. Literature data showed that Lys replacement with Orn maintained or even enhanced the inhibitory activity, while replacements with Dab and Dap decreased inhibitory activity [5]. This correlated with our results showing no analgesic effect of Dab- and Dap-N/OFQ(1-13)NH₂-analogues compared to nociceptin itself. [Dap⁹]N/OFQ(1-13)NH₂ alone showed

slight analgesic effect compared to the control on the 10th min after its administration.

In a second experiment, in order to investigate the involvement of opioidergic system in analgesic effects of the analogues with the most pronounced ones versus control, the non-competitive opioid-receptor antagonist naloxone was used. The obtained results showed that analgesic effects of the Orn-analogues and [Dap⁹]N/OFQ(1-13)NH₂ were naloxone-reversible (Fig. 2).

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 [1, 2]. Considering these findings, it was not surprising that naloxone inhibited the analgesic effects of newly synthesized NO/FQ (1-13)-analogues which suggested the involvement of opioidergic system. The effect was more pronounced for [Orn¹³]N/OFQ(1-13)NH₂ (Fig. 2).

In conclusion, our results suggest:

- the analogues with Orn instead of Lys at the 13th position have a more pronounced analgesic effect than NO/FQ (1-13);
- the analogues with Orn instead of Lys at the 9th position express a significantly less pronounced analgesic effect, compared to NO/FQ (1-13);
- the opioidergic system is involved in the analgesic effects of the newly synthesized NO/FQ (1-13)-analogs.

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