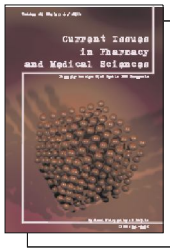

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Analgesic effects of deltorphin analogues EW1 and EW2 in tail-immersion test in mice

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ABSTRACT

The aim of the study was to evaluate whether EW1 and EW2, the newly synthesized analogues of deltorphin, a highly potent mu- (MOP) and delta-opioid receptors (DOP) ligand, induce antinociceptive effects in the tail-immersion test after intracerebroventricular (i.c.v.) administration. Our study indicates that these peptides, administered at the dose of 20 nmol, exert stronger or comparable antinociceptive effects as those exerted by morphine (13 nmol). A more detailed study indicated that β-funaltrexamine (β-FNA) – a MOP antagonist – very strongly and, to the lower extent than naltrindole (NTI), a DOP antagonist, inhibited the antinociceptive effects of peptides, observed in the tail-immersion test. Nor-binaltorphimine (nor-BNI), a kappa-opioid receptor (KOP) antagonist, did not influence that effect. Those data indicated an involvement of both types of opioid receptors, MOP and DOP, in the antinociceptive effects of the peptides with a dominant role of MOP.

Keywords:deltorphin analogues, morphine, nociception, tail-immersion

INTRODUCTION

The three classes of opioid receptors, namely MOP, DOP and KOP, are major receptors for analgesia and are expressed at central and peripheral sites within the pain control circuits. Opioid receptors are also largely distributed in other neural pathways, where they regulate reward and affective states [14,16,17]. Morphine (MOP agonist), a principal drug of the opioid family, is still the most important agent, used for alleviation of severe pain [26]. However, morphine administration is associated with a number of problematic side-effects, such as tolerance, dependence, constipation, addiction liability and opioid-induced hyperalgesia [24]. KOP agonists also are known to provide some analgesic properties, as well as dysphoria, which severely limits their usefulness. Nevertheless, DOP agonists represent a potentially useful alternative target in the treatment of pain, as it may result in fewer side effects and lower abuse potential [2]. Therefore, DOP agonists remain potentially important therapeutic targets for the development of novel analgesic compounds.

Natural deltorphins are linear heptapeptides secreted by the skin glands of Phyllomedusa amphibians with higher affinity and selectivity for DOP than any other known endogenous compound [4, 13]. Pharmacological studies have demonstrated that deltorphin I and deltorphin II are potent opiate agonists that stimulate locomotor activity and stereotyped behaviors in rats [15], improve memory consolidation in mice [18] and activate immunocytes in humans and invertebrates [23]. However, the majority of research projects focus on the antinociceptive properties of these compounds. It is particularly interesting in view of their higher antinociceptive activity in inflammatory or neuropathic pain [2,8,11], while with fewer side effects than MOP agonists.

In the current studies, the antinociceptive potential of newly synthesized deltorphin derivative peptides – EW1 and EW2 (Fig. 1) – were assessed in mice in the tail-immersion test. The antinociceptive effects of peptides were compared to morphine effects. In order to determine the respective contribution of MOP, DOP and KOP in the antinociceptive effects of EW1 and EW2, the selective antagonists of opioid receptors were used to antagonize the effect of the peptides in the tail-immersion test.

MATERIALS AND METHODS

The experiments were carried out on male Swiss mice (HZL, Warsaw, Poland). The animals were maintained under standard laboratory conditions (12-h light/dark cy-

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cle, temperature: 21±1°C) with free access to tap water and laboratory chow (Bacutil, Motycz, Poland). They were adapted to laboratory conditions for, at least, 1 week. Each experimental group consisted of 8–25 animals. All the experiments were carried out in line with the National Institute of Health Guidelines for the care and use of laboratory animals and with provisions of the European Council Directive of 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the Local Ethics Committee 24/13.

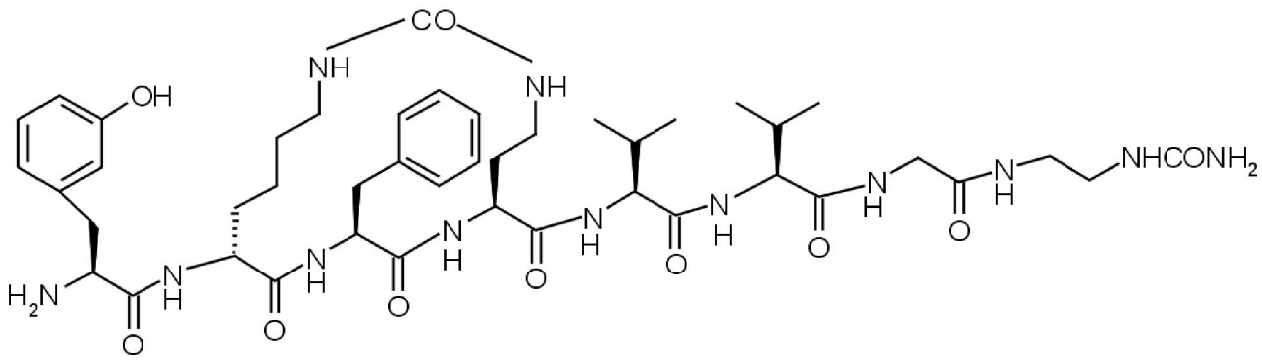
Drugs and injection procedure. Both analogues of deltorphin were synthesized at the Laboratory of Peptides, Department of Chemistry, Warsaw University, Poland (Fig. 1). The peptides were dissolved in physiological saline (0.9% NaCl) and injected i.c.v. at the dose of 20 nmol and in volume of 5 µl. Naltrindole hydrochloride (NTI, 5 nmol), β-funaltrexamine hydrochloride (β-FNA, 5 nmol), and nor-binaltorphimine hydrochloride (nor-BNI, 10 nmol) were purchased from Tocris Cookson Ltd. (Bristol, UK). Those opioid antagonists were each time freshly prepared immediately before experiments as isotonic saline solutions, and were given i.c.v. in 5 µl volumes. The control group received saline injections of the same volume and via the same route.

The i.c.v. injections were performed, following the method described by Haley and McCormick [6]. In brief, peptide solution was loaded into a 10 µl syringe. A mouse was hand-held and gently restrained, the skull was punctured perpendicularly to the dorsal surface and 5 µl of the solution was injected into the lateral ventricle. All the solutions were slowly i.c.v. injected for a period of 30 s. The injection site was 1.5 mm from the middle, 1 mm from the bregma and 3 mm from the surface of the skull. Mouse skull is sufficiently soft to enable needle insertion with a minimal force. The procedure takes less than a minute and requires no anesthetics, surgery, or incision. The correctness of the i.c.v. injections was histologically verified after the experiments, using cresyl violet. Approximately 10–15% of the animals indicated incorrect injections and were withdrawn from the experiments.

Tail-immersion test. The tail-immersion test was carried out, as described by Janssen et al. [10]. In order to determine nociceptive reaction, the animal tails were placed in a water bath, heated to 52°C, and the latency of

EW1 {H-Tyr-D-Lys(&[1])-Phe-Dab(&[2])-Val-Val-Gly-$NHCH_2CH_2NHCONH_2$][&[1]CO&[2]]} $C_{44}H_{66}O_{10}N_{12}$

EW1



EW2 {H-Tyr-D-Lys(&[1])-Phe-Orn(&[2])-Val-Val-Gly-$NHCH_2CH_2NHCONH_2$][&[1]CO&[2]]} $C_{45}H_{68}O_{10}N_{12}$

EW2

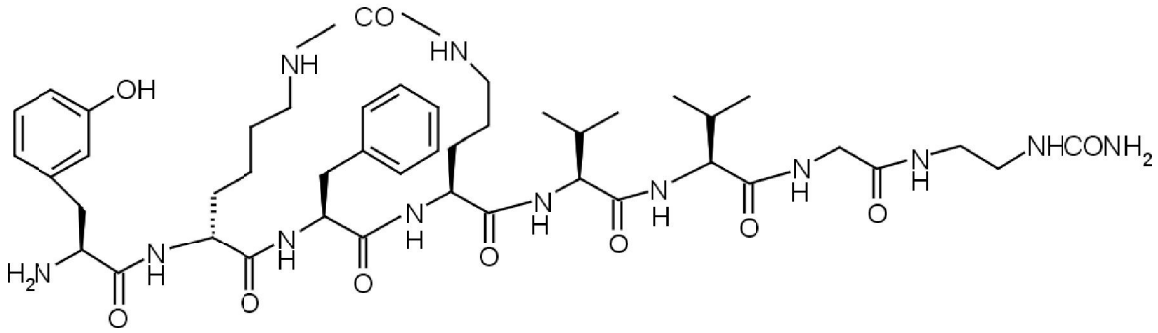


Fig. 1. Fig. 1. Structure of the EW1 and EW2

response (in s; reflexive withdrawal of the distal half of the tail after its immersion in water) was measured before injections of the drugs (baseline latency response) and at 15 min intervals for subsequent 60 min, and then at 30 min intervals, up to 120 min (post-treatment latency response) after drug injections. The cut-off time of 20 s was set to prevent tail skin tissue damage. Morphine antinociception was induced by i.c.v. injections of morphine hydrochloride at the dose of 13 nmol. In order to examine an antinociceptive effect of EW1 and EW2, the peptides were i.c.v. injected at the dose of 20 nmol. The control group received saline instead of either morphine or the peptide. β-FNA – a selective MOP antagonist (5 nmol, 24 h before peptide injection [7]), NTI – a DOP antagonist (5 nmol, 5 min before peptide injection [19]), and nor-BNI, a KOP antagonist (10 nmol, 1 h before peptide injections [25]) were administered to evaluate MOP, DOP and KOP contribution levels in the EW1– and EW2-induced antinociception.

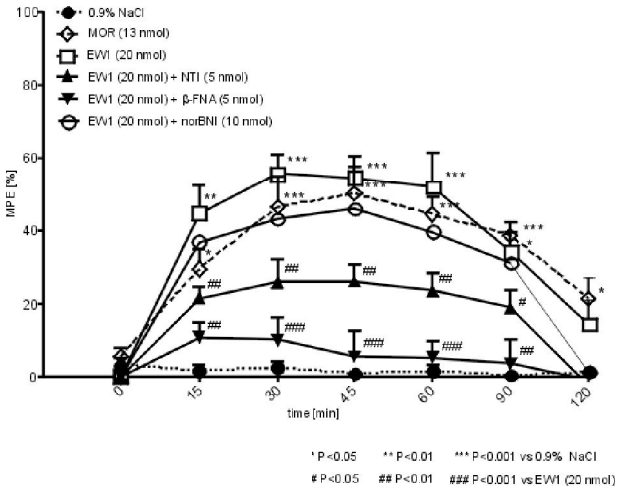


Fig. 2. The influence of opioid antagonists: ß-FNA (5 nmol, i.c.v., 24 h before test), NTI (5 nmol, i.c.v., 5 min before test), and nor-BNI (10 nmol, i.c.v., 1 h before test) on EW1 (20 nmol, i.c.v.) induced antinociception in the tail-immersion test in mice. Statistical analysis was performed using two-way ANOVA followed by the Bonferroni post hoc test. The results are expressed as a mean ± SEM (N = 6–10). *P < 0.05, **P < 0.01, ***P < 0.001 vs. saline; #P < 0.05, ##P < 0.001, ###P < 0.001 vs. EW1

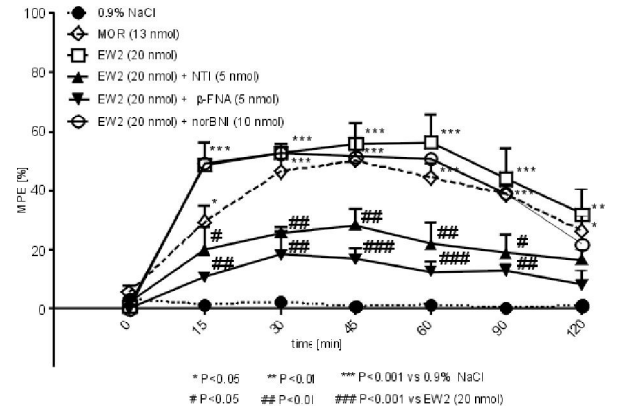


Fig. 3. The influence of opioid antagonists: ß-FNA (5 nmol, i.c.v., 24 h before test), NTI (5 nmol, i.c.v., 5 min before test), and nor-BNI (10 nmol, i.c.v., 1 h before test) on EW2 (20 nmol, i.c.v.) induced antinociception in the tail-immersion test in mice. Statistical analysis was performed using two-way ANOVA followed by the Bonferroni post hoc test. The results are expressed as a mean ± SEM (N = 6–10). *P < 0.05, **P < 0.01, ***P < 0.001 vs. saline; #P < 0.05, ##P < 0.001, ###P < 0.001 vs. EW2.

Statistical analysis. Data are presented as means ± SEM and expressed as percent of possible maximum effect (MPE%) calculated as: MPE(%) = 100 × [(post-drug response – baseline response)/(cut-off response – baseline response)]. Behavioral time course data were analyzed, using a two-way ANOVA (followed by the Tukey–Kramer post hoc test). Any value of P 0.05 was considered statistically significant (GraphPad Prism 5.0, GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

EW1 injections (20 nmol, i.c.v.) dose-dependently increased tail-immersion latency, reaching a maximal antinociceptive response in 30 min after injection in mice (see Fig. 2). The two-way ANOVA revealed significant effects for both dose [F(5, 256) = 25.34, P<0.001] and time [F(6, 256) = 14.76, P<0.001]. Co-injection of β-FNA (5 nmol, i.c.v., a MOP antagonist), more effectively than NTI, the DOP antagonist (5 nmol, i.c.v.), blocked the EW1-induced antinociception (20 nmol, i.c.v.). However, nor-BNI, the KOP antagonist (10 nmol, i.c.v.) did not modify tail-immersion latency induced by i.c.v. administration of EW1 (20 nmol).

When compared with vehicle-treated animals, the i.c.v. injections of EW2 (20 nmol) significantly increased tail-immersion latency, reaching a maximal antinociceptive response in 60 min after injection in mice (see Fig. 3). The two-way ANOVA of those data revealed significant effects for both dose [F(5, 224) = 47.4, P<0.0001] and time [F(6, 224) = 14.76, P<0.0001]. A combined injection with β-FNA (5 nmol, i.c.v.) slightly more effectively than NTI (5 nmol, i.c.v.) reduced EW2-induced antinociception (20 nmol, i.c.v.). Similarly to results for EW1, nor-BNI, the KOP antagonist (10 nmol, i.c.v.), did not change EW2-induced tail-immersion latency.

Generally, both deltorphin analogues, at the dose of 20 nmol, induced stronger antinociceptive effect than morphine at the dose of 13 nmol (Fig. 2 and 3).

DISCUSSION

The antinociceptive effects of opioids results from their interactions with MOP, DOP or KOP [3]. All these receptors are represented in areas, associated with pain modulation, including: the periphery, the spinal cord dorsal horn, the brainstem, the thalamus and the cortex, where they embody a pain transmission suppression system [9]. The present study demonstrated that both synthetic

analogues of deltorphin, i.e., EW1 and EW2, were highly potent analgesics after their i.c.v. injection in the tail-immersion test. In that test, EW2, administered i.c.v. at the dose of 20 nmol, induced antinociceptive effects approximately similar to those of morphine (13 nmol, i.c.v.), whereas EW1 (20 nmol, i.c.v.) was more effective than morphine. The observed effect was achieved in 15 min after injection with a maximum effect after 30 and 60 minutes for EW1 and EW2, respectively. The antinociceptive effect remained stable for up to 2 h after injection. Those long-lasting antinociceptive effects may be suggestive of a higher resistance of the peptides to enzymatic degradation vs. natural deltorphins.

A more detailed study with co-administration of selective opioid receptor antagonists indicated that the antinociceptive effect of EW1 and EW2 was significantly inhibited by DOP antagonist (NTI) or MOP antagonist (β-FNA), however, â-FNA showed a stronger inhibitory effect on EW1-induced than on EW2-induced analgesia. The KOP antagonist nor-BNI was ineffective in blocking the antinociceptive effects of those peptides. Those effects suggest that both peptides are mixed MOP/DOP agonists *in vivo* and that their antinocieptive effects are mediated by an interaction between DOP and/or MOP. Interactions between MOP and DOP were suggested ealier [22], as well as colocalization of those receptors, e.g., at the same axonal terminals of the superficial dorsal horn [1]. Taken together, the evidence for colocalization of MOP and DOP may result in formation of heterodimers that could modulate opioid function in a different way vs. monomers [5]. Although other authors [21] suggest a minimal possibility for such heterodimerization in nociceptors, they do not preclude their effects in the central nervous system and, additionally, receptor dimerization is considered to be a potential mechanism to modulate opioid function [20].

Nevertheless it appears that MOP predominates in the antinociceptive action of EW1, and both types of opioid receptors play an equivalent role in EW2 effects. It seems that MOP-DOP interactions potentiated the peptide-induced antinociception in comparison with the effects of morphine – a MOP agonist.

CONCLUSIONS

Summing up, our study indicates that EW1 and EW2, a new synthetic analogues of deltorphin, given supraspinally, induce strong antinociceptive effects in the tail-immersion test. A more detailed study (with antagonists of opioid receptors) suggested that both compounds were mixed MOP/DOP agonists with a dominant role of MOP in their antinociceptive effect. Current study confirms our prior results which showed that deltorphin analogs produced comparable but stronger antinociceptive effect than morphine (13 nmol) [12].

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