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Endomorphin-1 analogs with oligoarginine-conjugation at C-terminus produce potent antinociception with reduced opioid tolerance in paw withdrawal test

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ABSTRACT

For clinical use, it is essential to develop potent endomorphin (EM) analogs with reduced antinociceptive tolerance. In the present study, the antinociceptive activities and tolerance development of four potent EM-1 analogs with C-terminal oligoarginine-conjugation was evaluated and compared in the radiant heat paw withdrawal test. Following intracerebroventricular (i.c.v.) administration, all analogs 1–4 produced potent and prolonged antinociceptive effects. Notably, analogs 2 and 4 with the introduction of D-Ala in position 2 exhibited relatively higher analgesic potencies than those of analogs 1 and 3 with β-Pro substitution, consistent with their μ-opioid binding characteristic. In addition, at a dose of 50 μmol/kg, endomorphin-1 (EM-1) failed to produce any significant antinociceptive activity after peripheral administration, whereas analogs 1–4 induced potent antinociceptive effects with an increased duration of action. Herein, our results indicated the development of antinociceptive tolerance to EM-1 and morphine at the supraspinal level on day 7. By contrast, analogs 1–4 decreased the antinociceptive tolerance. Furthermore, subcutaneous (s.c.) administration of morphine at 50 μmol/kg also developed the antinociceptive tolerance, whereas the extent of tolerance developed to analogs 1–4 was largely reduced. Especially, analog 4 exhibited non-tolerance-forming antinociception after peripheral administration. The present investigation gave the evidence that C-terminal conjugation of EM-1 with oligoarginine vector will facilitate the development of novel opioid analgesics with reduced opioid tolerance.

1. Introduction

Neuropeptides have been indicated as primary regulatory molecules in cellular and intercellular physiological responses, and possess great promise for the treatment of several central nervous system (CNS) disorder by acting through their special receptors [1]. Opioid peptides, acted as neurotransmitters and neuromodulators, have the potential to be pharmaceutical agents for the treatment of pain owing to their elevated potency and centrally mediated actions of pain processes [2,3]. More recently, two endogenous neuropeptides named endomorphin-1 (EM-1) and endomorphin-2 (EM-2) were firstly isolated from bovine brain and the human cortex by Zadina et al. [4]. Both endomorphins (EMs) exhibited high affinity and selectivity for μ-opioid receptor. Centrally administered EMs produced potent antinociception with less side effects than opioid alkaloids, including reduced cardio-respiratory depression [5], reward behavior [6] and locomotor activity [7]. Therefore, EMs have the potential to be pharmaceutical agents for the treatment of pain. However, the clinical applications of EMs remain unsuccessful due to their low metabolic stability and inability to cross the blood-brain barrier (BBB) [8].

Till now, numerous approaches have been devised in an attempt to improve the CNS delivery of EMs [9–11]. Recently, cell-penetrating peptides (CPPs) such as SynB and oligoarginine are considered as useful vectors for the intracellular and brain delivery of therapeutic molecules by a receptor-independent mechanism. It was reported that conjugation of EM-1 to SynB3, an efficient CPP-carrier, via amide, maleimide and

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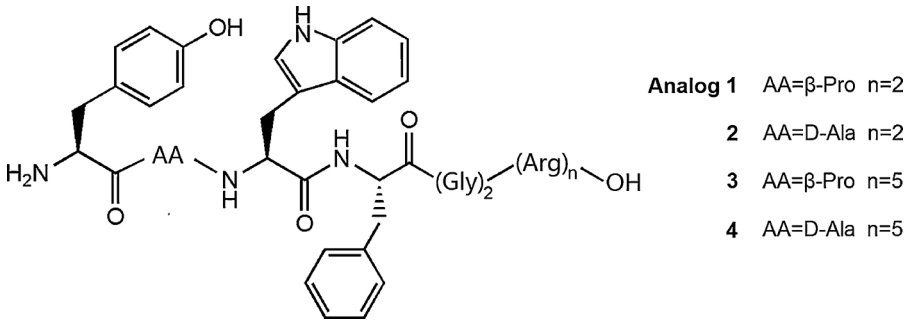

Fig. 1. Schematic diagram of EM-1 and its analogs 1–4 investigated in this study.

disulfide linkages improved its BBB permeability [12]. In our previous study [13], we have demonstrated that novel EM-1 analogs with C-terminal linked to oligoarginine vector, a kind of cationic cell-penetrating peptides, produced potent antinociception after peripheral administration. Moreover, we found that combined modifications of EM-1 with unnatural amino acid substitutions and oligoarginine-conjugation gave an efficient strategy to improve analgesic profile of EM-1 with reduced gastrointestinal side effects [14].

Tolerance is one of the common complications of opioid treatment, leading to a gradual loss of analgesic potency and efficacy, and reduced duration of action [15]. It is widely accepted that the activation of μ-opioid receptor is responsible for the emergence of antinociceptive tolerance. As potent μ-opioid receptor ligands, EMs have been shown to induce antinociceptive tolerance [16–18]. It is essential to develop new EMs analogs with reduced tolerance for clinical therapy. Therefore, to increase our knowledge, the present study was undertaken to investigate the antinociceptive activities and tolerance development of four potent EM-1 analogs with oligoarginine-conjugation at C-terminus in the radiant heat paw withdrawal test after central and peripheral administration.

2. Materials and methods

2.1. Animals and drugs

Male Kunming mice were used in this study. Animals were housed in an animal room that was maintained at 22 ± 2 °C with a 12-h light: 12-h dark cycle, and given free access to food and water. All animals were cared for and experiments were carried out in accordance with the principles and guidelines of the Ethics Committee of Harbin Institute of Technology.

The peptides EM-1 and its analogs 1–4 (analog 1, Tyr-β-Pro-Trp-Phe-$(Gly)_2$- $(Arg)_2$-OH; analog 2, Tyr-D-Ala-Trp-Phe-$(Gly)_2$-$(Arg)_2$-OH; analog 3, Tyr-β-Pro- Trp-Phe-$(Gly)_2$-$(Arg)_5$-OH; analog 4, Tyr-D-Ala-Trp-Phe-$(Gly)_2$-$(Arg)_5$-OH) used in this study were synthesized by manual solid-phase synthesis method and characterized by reversed-phase HPLC and electrospray ionization-mass spectrometry as described in our previous study [14]. The purities of all the peptides were more than 95%. Morphine hydrochloride was purchased from Shenyang First Pharmaceutical Factory, China. All compounds were dissolved in saline and stored at −20 °C.

2.2. Implantation of cannula into ventricle for intracerebroventricular (i.c.v.) administration

The surgical implantation of cannula was conducted in an aseptic environment. Male Kunming mice weighing 18–22 g were anesthetized with 60 mg/kg pentobarbital sodium by intraperitoneal (i.p.) injection and placed in a stereotaxic apparatus. The incision area of the scalp was shaved, and a sagittal incision was made in the midline, exposing the surface of the skull. A single hole was drilled through the skull at 2.5 mm posterior and 1 mm lateral from the bregma. A stainless steel guide cannula was implanted 3 mm ventral from the surface of the skull for i.c.v administration, and fixed to the skull using dental cement. To prevent occlusion, a dummy cannula was inserted into the guide cannula. The dummy cannula protruded 0.5 mm from the guide cannula. After surgery, the animals were allowed to recover for at least 4 days. During this time, mice were gently handled daily to minimize the stress associated with manipulation of the mice throughout the experiments.

2.3. Drug administration

I.c.v. administration was performed in conscious mice following the method previously described [19]. Drugs were injected in a volume of 4 μl at a constant rate of 10 μl/min, followed by 1 μl of saline to flush in the drug using a 25-μl microsyringe. Vehicle control animals received appropriate saline. After completion of behavioral test, the proper injection site was verified in pilot experiments by administration and localization of methylene blue dye. Only the data from those animals with dispersion of the dye throughout the ventricles were used in this study. For peripheral administration, mice received a subcutaneous (s.c.) injection at a volume of 100 μl drugs or saline.

2.4. Radiant heat paw withdrawal test

The nociceptive response was assessed by the radiant heat paw withdrawal test using PL-200 radiant heat apparatus (Chengdu Taimeng Technology & Market Corporation, China). Each mouse was adapted to the testing environment for at least 30 min before experiment. The radiant heat source was positioned under the transparent floor directly beneath the hind paw. The light beam focused on the plantar surface of the hind draw, and the latency for paw withdrawal response against radiant heat stimulation was measured. The intensity of radiant heat was adjusted so that the control latency (CL) for paw withdrawal response was 3–5 s before drugs administration. The mice were test again after i.c.v. or s.c. administration of drugs at different times. The test latency (TL) was then recorded with a maximum cut-off scored of 10 s to minimize the tissue damage. The antinociception was calculated as percentage of maximal possible effect (%MPE), expressed in the following manner: %MPE of aninociception = 100 × (TL − CL)/(10 − CL).

2.5. Development of antinociceptive tolerance

To determine the antinociceptive tolerance development, mice received i.c.v. or s.c. administration of either saline or drugs once daily for 7 days, between 10 and 12 a.m. [20]. The development of antinociceptive tolerance was established by assessing the antinociceptive effects following i.c.v. or s.c. administration of drugs.