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Endomorphin-1 analogues (MELs) penetrate the blood–brain barrier and exhibit good analgesic effects with minimal side effects

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ABSTRACT

Endomorphins are endogenous opioid peptides in mammals and display a strong antinociceptive effect after central administration. However, the clinical usage of these peptides is limited because of their diminished analgesic effect following systemic injection and their inability to cross the blood–brain barrier. In this study, we characterized the *in vivo* effects of four novel endomorphin-1 analogues (termed MELs), which previously showed potential as highly potent analgesics with a good pharmacological profile *in vitro*. The analogues were administered intravenously to several rodent pain models to examine their antinociception and blood–brain barrier permeability. The tested peptides, especially MEL1214, showed good analgesic activity and blood–brain barrier permeability. Behavioral studies showed dose-dependent analgesic effect after systematic administration of MEL1214 in the tested pain models. Pre-treatment of subcutaneous administration of naloxone methiodide did not affect the antinociception of these peptides. As compared to morphine, MEL1214 was less prone to induce tolerance after consecutive intravenous administration for 5 days. Gastrointestinal transit was evaluated by the isolated colon response and bead expulsion to determine the potential constipation effect. In contrast to morphine, MEL1214 produced no significant constipation effect at an equivalent dose. MEL1214 shows promise as a suitable compound to treat pain with reduced side effects, and exhibits good potential to be further developed as a novel opioid analgesic in pain treatment.

1. Introduction

Opioid therapeutics have been used as analgesic drugs for thousands of years to relieve chronic and acute pain. Each year, millions of patients are prescribed opioid analgesics as part of their medical therapy for pain relief (Kanjhan, 1995; Christo and Mazloomdoost, 2008; Parsells Kelly et al., 2008). However, the clinical usage of these drugs is limited because of their well-known side effects such as tolerance, addiction, constipation, respiratory depression, and loss of consciousness (Kieffer and Gaveriaux-Ruff, 2002; Pradhan et al., 2012). Endogenous opioid peptides that target opioid receptors are potential alternatives to exogenous opioids for pain relief (Gentilucci, 2004). In particular, μ-opioid receptor (MOR) agonists are considered to provide outstanding benefits for the relief of severe pain (Janecka et al., 2010).

Endomorphin-1 (EM-1, H-Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (EM-2, H-Tyr-Pro-Phe-Phe-NH_2) are endogenous opioid peptides, which were isolated from the human cortex and the bovine brain (Zadina et al., 1997). These two peptides show the highest affinity and selectivity toward MOR among all known endogenous opioid peptides (Zadina et al., 1997; Fichna et al., 2007), and have a favorable therapeutic profile relative to other μ–opioids by reducing the pain response without introducing the side effects associated with morphine use (Czapla et al., 2000; Wilson et al., 2000). The rewarding behavior of EMs could be separated from their antinociception (Wilson et al., 2000), and they are less prone to induce cardiovascular disorders and respiratory depression (Czapla et al., 2000). However, the clinical development of EMs has been hampered by their poor metabolic stability and limited ability to penetrate the blood–brain barrier (BBB)

Abbreviation: BBB, blood–brain barrier; cAMP, cyclic adenosine monophosphate; CCI, chronic constriction injury; CNS, central nervous system; DOR, δ opioid receptor; EM-1, endomorphin-1; EM-2, endomorphin-2; EC_{50}, half-maximal effective concentration; E_{max}, maximal effective concentrations; ERK, extracellular regulated kinases; i.c.v., intracerebroventricular; i.p., intraperitoneally; i.v., intravenous; KOR, κ opioid receptor; MOR, μ opioid receptor; MPE, maximum possible effect; s.c., subcutaneous; WGT, whole gut transit.

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(Mentlein, 1999; Shane et al., 1999; Tomboly et al., 2002; Liu and Wang, 2012). A major limitation to the use of EMs as painkillers is their low stability against enzymatic degradation. Natural EMs undergo rapid degradation when they move into the functional tissue and compete for receptor binding. As a result, the physiological activities of exogenously administered EMs are low to nonexistent. Furthermore, neither of these peptides can cross the BBB to reach the central nervous system (CNS), and thus display limited potential for clinical pain treatment. In an attempt to overcome the shortcomings associated with the bioavailability of EMs, many strategies have been developed to improve their pharmacological profiles, including cationization, glycosylation, lipidization, structural restriction, and substitution with unnatural amino acids.

When attempting to maintain the high potency of an analgesic, the impact of side effects should not be ignored. In most instances, a repeated and high dose of analgesics is often administered in order to dampen the patient's pain, which could cause adverse side effects such as tolerance and constipation (Marderstein and Delaney, 2008; Schneider and Kirsh, 2010). Tolerance to opioids usually results in a decrease of the antinociceptive effect, leading to a requirement for a higher dose to maintain a consistent response (Collett, 1998; Kelly, 2013; Varamini et al., 2013a). Constipation is another common side effect of opioids, since opioids decrease peristaltic activity in the gastrointestinal tract (Kurz and Sessler, 2003; Sternini et al., 2004; Wade et al., 2012). Activation of MOR in the gut would cause opioid–induced constipation, and some patients are forced to reluctantly discontinue adequate pain treatment because of this distressing side effect (Camilleri, 2011; Bader et al., 2013; Ford et al., 2013).

In our preliminary study, we reported a novel series of EM-1 analogues, designated MELs, which were obtained by introduction of unnatural β-amino acids, α-methylene-β-amino acids (Maps), into the 4th position of EM-1 as shown in Fig. 1 (Wang et al., 2012). These analogues displayed high bioactivity toward MOR, with the most active analogue exhibiting about 10-fold better affinity to MOR than the parent peptide. The agonistic properties of the MELs were confirmed based on ERK1/2 phosphorylation and cAMP accumulation assays in HEK293 cells. The MELs also displayed increased stability and enhanced antinociceptive activity in the warm water tail-flick assay after intracerebroventricular (i.c.v.) administration as compared with EM-1. These features suggest that MELs are ideal lead compounds that should facilitate the discovery of novel clinically effective analgesic drugs with minimal side effects.

Here, in an effort to evaluate the potential of the MELs as new analgesic drugs, the following four compounds were examined (Fig. 1), H-Tyr-Pro-Trp-(Ph)Map-NH_2 (MEL1201), H-Tyr-Pro-Trp-(3-ClPh)Map-NH_2 (MEL1209), H-Tyr-Pro-Trp-(2-furyl)Map-NH_2 (MEL1214), and H-Tyr-Pro-Trp-(3-furyl)Map-NH_2 (MEL1224). Their antinociceptive activities were evaluated at different doses in four animal pain models (acute, inflammatory, visceral, and neuropathic pain) after peripheral intravenous (i.v.) administration in order to confirm their *in vivo* stability and BBB permeability. The propensity of the selected MELs to induce tolerance after repeated administration was also studied. Furthermore, as constipation is a major problem of clinical opioid therapy, the actions of MELs on gastrointestinal functions were assessed by *in vivo* assays.

2. Materials and methods

2.1. Animals

Animals (Animal Center of Medical College of Lanzhou University, Gansu, People's Republic of China) were housed in a temperature-controlled environment (22 ± 1 °C) under standard 12 h light/dark conditions and received food and water ad libitum. Animals were used only once and received good care and humane treatment. The results of all studies involving animals are reported in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the local ethics committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Chemicals

In the present study, EM-1 and its analogues were synthesized by manual solution-phase methods as described in our previous report (Wang et al., 2012; Liu et al., 2013). Crude products were purified by semipreparative RP-HPLC and were 95–99% pure as determined by analytical RP-HPLC. The molecular weight of the peptide was confirmed by an electrospray ionization mass spectrometer (ESI-Q-TOF Maxis-4G, Bruker Daltonics, Germany). In addition, naloxone hydrochloride, naloxone methiodide and formalin were purchased from Sigma–Aldrich (St. Louis, MO, USA). All compounds were dissolved in saline solution and stored at −20 °C.

2.3. Warm water tail-flick test

The nociceptive response was assessed by the warm water tail-flick test, as described previously (Wang et al., 2012). Briefly, male Kunming mice weighing 18–22 g were employed, various dose of analogues were injected i.v. and the warm water tail-flick responses were measured at different times. For the study involving the opioid antagonist, animals were pretreated with naloxone and naloxone methiodide before i.v. challenge with peptides. Nociception was evoked by immersing the mouse tail in warm water (50 ± 0.2 °C) and measuring the latency to withdrawal. Before treatment, each mouse was recorded the control latency (CL) to tail-flick, and those with a latency of approximately 3–5 s were selected. The latency to tail-flick was defined as the test latency

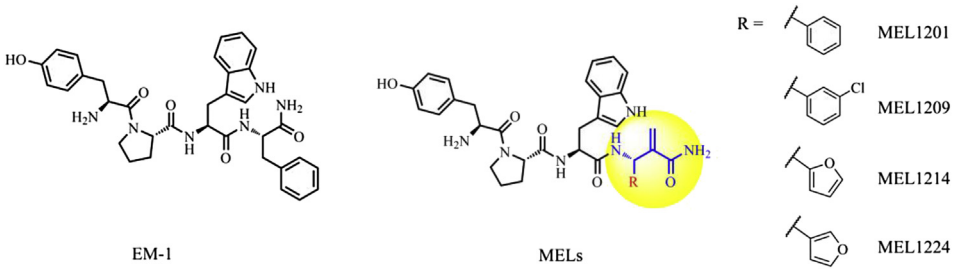

Fig. 1. Structure of EM-1 and its analogues MEL1201, MEL1209, MEL1214 and MEL1224. The Map[4] substitution is indicated in yellow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)