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Peripherally acting novel lipo-endomorphin-1 peptides in neuropathic pain without producing constipation

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ABSTRACT

We previously described two novel analogues of endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2, **1**), modified with an 8-carbon lipoamino acid (C8LAA) with or without replacement of Tyr[1] with 2,6-dimethyltyrosine (Dmt) at the N-terminus of the peptide (compounds **3** and **4**, respectively). They were shown to be more stable and permeable, and acted as potent μ-opioid receptor agonists. In this study we report that the C8LAA modification resulted in successful systemic delivery of both analogues. They produced potent dose-dependent pain relief in a chronic constriction injury-rat model of neuropathic pain after intravenous administration with ED_{50} values obtained at 6.58 (±1.22) μmol/kg for **3** and 6.18 (±1.17) μmol/kg for **4**. Using two different rat models of constipation that assess the effects of μ-opioid receptor agonists on stool hydration and gastro-intestinal motility, compound **3** produced insignificant constipation at 16 μmol/kg, whereas morphine elicited significant constipation at 2 μmol/kg. Compound **3** in contrast to morphine, did not attenuate the hypercapnic ventilatory response at 5 μmol/kg, a dose that fully alleviated hindpaw sensitivity at the time of peak effect in CCI-rats. This finding revealed the lack of respiratory depression effect at antinociceptive dose.

1. Introduction

Pain can arise from tissue damage which consequently activates primary nociceptive afferents.[1] On the other hand, pain may be due to secondary neuroplastic alterations in the nociceptive system that can arise from a nerve lesion or dysfunction in the peripheral nervous system (PNS) or central nervous system (CNS).[2] This type of pain has been defined as neuropathic pain according to The International Association for the Study of Pain (IASP).[3] Regardless of its origin, management of neuropathic pain is considered a great challenge to clinicians as a result of various limitations in the use of currently available drugs.[4] Even opioids which are effective in nociceptive pain reportedly lack potent analgesic efficacy in neuropathic pain[5,6] or are only effective at high doses.[7] Alternatively, the recently discovered endogenous ligands of μ-opioid (MOP) receptors, endomorphins, were shown to be greatly effective following central administration in rodent neuropathic pain models in animals.[6] Following intravenous administration, they have been found to induce cardio-respiratory adverse effects only at high doses.[8] Intracerebroventricular (i.c.v.) administration of endomorphin-1 produces potent antinociception without eliciting reward behaviour while morphine has reward-related properties.[9] Unlike morphine, no locomotor activity has been observed after administration of endomorphins by the i.c.v. route.[10] However, endomorphins, in common with other small peptides, suffer from low metabolic stability and an inability to penetrate the gut-blood or blood–brain-barrier (BBB), which prevent their clinical use. Even after central administration in animal models, endomorphins produced a very short duration of antinociception (less than 15 min).[11] Hence, chemical modification of these endogenous opioid peptides is essential to overcome these limitations.

In a previous investigation, we described two novel endomorphin-1 analogues, bearing 2-aminooctanoic acid (an 8-carbon lipoamino acid, C8LAA) on the N-terminus with/without substitution of Tyr[1] by the non-natural amino acid, 2,6-dimethyl tyrosine (Dmt). Dmt was previously shown to enhance the receptor binding affinity and potency of several opioid peptides[12] including endomorphin-1.[13] These analogues were found to be significantly more stable and more permeable than endomorphin-1 across Caco-2 cell monolayers with comparable or higher binding affinity and agonist activity at MOP receptors.[14] To examine if lipid modification of endomorphin-1 results in systemically active peptides, the pain modulating activity of the C8LAA-modified analogues of endomorphin-1 (compound **3**) and Dmt-endomorphin-1 (compound **4**) was evaluated in a widely-utilized rat model of neuropathic pain

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following intravenous (i.v.) administration. Endomorphin-1 (**1**) and the Dmt-substituted analogue (**2**) were also tested for their antiallodynic activity after iv administration.

Constipation is one of the most common adverse effects associated with the use of opioid analgesics and it is mediated by both a central opioidergic mechanism as well as local opioid receptors in the gastrointestinal (GI) tract. As they are MOP receptor agonists, i.c.v. endomorphins reduce GI motility inhibiting gastric emptying and intestinal propulsions.[15,16] Respiratory depression is another side effect caused by many opioid analgesics. Intravenously administered endomorphins attenuate hypercapnia-induced stimulation of ventilator responses at doses that are much higher than those that produce analgesia in contrast to morphine and other opioids such as DAMGO ([D-Ala_2, N-$MePhe_4$, Gly-ol]-enkephalin).[8,17] In order to investigate if the C8LAA modification of endomorphin-1 has produced any alterations in the side effect profiles of the parent peptide, the constipation-inducing properties and respiratory depressant activities were also examined after intravenous bolus dose administration.

2. Results

2.1. Assessment of antiallodynia in CCI-rats

2.1.1. Wash-out rat protocol

The antiallodynic activity of endomorphin-1 (**1**) and derivatives **2**–**4** (structures shown in Figure 1) was evaluated in the chronic constriction injury (CCI)-rat model of neuropathic pain after iv administration of single bolus doses of test items. Compounds **1** and **2**, did not produce significant antiallodynia even at the highest tested doses in a manner analogous to vehicle as reported previously.[18] Figure 2A and B show ΔPWT versus time curves after administration of single iv bolus doses of compounds **3** and **4**, respectively. Both lipid-modified peptides significantly reversed allodynia in the ipsilateral hindpaw at a dose of 0.5 μmol/kg (Fig. 2C). These compounds produced dose-dependent antiallodynia (p <0.05 compared to vehicle) with a rapid onset of action at 15 min post-dosing and a duration of action of up to 2 h (Fig. 2A and B). The extent and duration of action of compounds **3** and **4** was insignificantly different for the relief of mechanical allodynia (Fig. 2C). The estimated mean (±SEM) ED_{50} values from the dose-response curves were 6.58 (±1.22) and 6.18 (±1.17) μmol/kg for compounds **3** and **4**, respectively (Fig. 3) and 2.64 (±1.37) μmol/kg for morphine. The calculated ED_{50} values for compounds **3** and **4** did not differ significantly (p >0.05). Therefore, due to the high cost and difficulties in producing Dmt required to synthesize compound **4**, compound **3** was used for further analysis as the more viable candidate for drug development studies. Consistent with expectations, single iv bolus doses of vehicle did not produce significant antiallodynia in the ipsilateral hindpaw showing that neither the dosing method nor the testing procedures contributed to the antiallodynic responses observed herein.

Interestingly, iv bolus doses of compound **3** and **4** (5 μmol/kg, respectively) produced insignificant antinociception in the contralateral (uninjured) hindpaw. On the contrary, morphine produced significant antinociception in the contralateral hindpaw of the CCI-rats at 2 μmol/kg (Fig. 4, p <0.001).

2.2. Drug-naive-rat protocol

To examine the effect of multiple dosing of the peptide analogues on the responsiveness of CCI-rats, three effective rising doses of compound **3** were tested on drug-naive CCI-rats. Compound **3** alleviated mechanical allodynia in the ipsilateral hindpaw at each dose in a manner similar to that produced in CCI-rats dosed with the same compound according to the 'wash-out' protocol where each rat received 3–4 single iv bolus doses of one test item or vehicle with each dose separated by a 2–3 day washout period. There was no significant difference between the effects produced by the corresponding doses in these two groups of rats (Fig. 5, p >0.05). The mean (±SEM) ED_{50} value was 6.64 (±1.34) μmol/kg for compound **3** in the drug-naive group of rats which was not significantly different from that obtained from rats administered compound **3** according to the wash-out protocol (p >0.05).

2.3. Assessment of constipation-inducing properties

2.3.1. Castor oil-induced diarrhoea test

There was insignificant inhibition of castor oil-induced diarrhoea up to 8 h post-dosing for compound **3** at 1.6 and 16 μmol/kg iv. The effects observed were not significantly different (p >0.05) from the effect observed in control animals administered vehicle. By contrast, single iv bolus doses of morphine at 2 μmol/kg significantly inhibited castor oil-induced diarrhoea (Fig. 6A, p <0.001).

2.4. Charcoal test

Following iv administration of compound **3** even at the highest dose, 16 μmol/kg, there was no significant effect on the gastrointestinal transit of the charcoal meal in contrast to morphine at 2 μmol/kg (Fig. 6B). The mean (±SEM) GI propulsion rate (%) was 77.0 (±2.0)% for the rats treated with compound **3** (n = 6) which was not significantly different (p >0.05) from that for vehicle-treated animals (65.3 (±3.1)%; n = 8). Conversely, morphine produced a marked decrease in gastrointestinal peristalsis (38.1 (±4.8)%, n = 8).

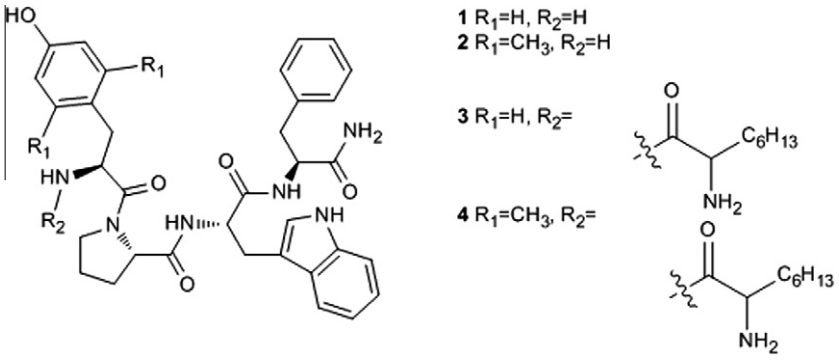

Figure 1. Endomorphin-1 (**1**) and the derivatives **2**–**4**.