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Antinociceptive effect of D-Lys^2, Dab^4 *N*-(ureidoethyl)amide, a new cyclic 1-4 dermorphin/deltorphin analog

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ABSTRACT

Background: A preliminary evaluation of antinociceptive activity of a new cyclic dermorphin/deltorphin tetrapeptide analog restricted *via* a urea bridge and containing C-terminal ureidoethylamid {[H-Tyr-D-Lys(&^{1})-Phe-Dab(&^{2})-$CH_2CH_2NHCONH_2$][&^{1}CO&^{2}]} (cUP-1) revealed a significant and long-lasting increase of pain threshold to thermal stimulation after systemic application. The current studies were aimed at further evaluation of cUP-1 activity in animal models of somatic and visceral pain. The influence of cUP-1 on motor functions was also investigated.

Methods: The influence of cUP-1 (0.5–2 mg kg^{-1}, *iv*) on nociceptive threshold to mechanical pressure and analgesic efficacy in formalin and acetic acid-induced writhing tests were estimated. The antinociceptive effect of cUP-1 was compared to that of morphine (MF). The influence of cUP-1 (1, 4 and 8 mg kg^{-1}, *iv*) on locomotor activity, motor coordination and muscle strength was estimated using open field and rota-rod tests and a grip strength measurement.

Results: Administration of cUP-1 in doses of 1 and 2 mg kg^{-1} elicited a significant increase of nociceptive threshold to mechanical pressure. MF applied in the same doses induced an antinociceptive effect only at the higher dose (2 mg kg^{-1}). There were no marked differences between the effect of cUP-1 and MF at each dose, at relative time points. In the writhing test and both phases of the formalin test, cUP-1 showed a significant, dose-dependent antinociceptive effect which did not markedly differ from that of MF. cUP-1 did not significantly affect motor functions of mice.

Conclusions: Systemic application of cUP-1 elicited a dose-dependent antinociceptive effect. The analgesic efficacy of cUP-1 on mechanical nociception, visceral and formalin-induced pain was comparable to that of MF. cUP-1 did not impair motor functions of mice.

Introduction

Morphine (MF) and synthetic opioid drugs acting mainly at μ opioid receptors (MOR) are still the mainstay of the therapy of severe acute (postoperative) and chronic (especially cancer) pain, but a number of serious adverse effects (*i.e.* on nervous, respiratory, cardiovascular, gastrointestinal, urinary systems) as well as development of tolerance and dependence often hinder this treatment or even make it impossible [1,2]. Therefore, continuous research efforts are focused on the development of new potent analgesics acting *via* opioid receptors but devoid of side effects due to therapy with classic MOR agonists.

In addition to endogenous opioid ligands in the mammalian nervous system, such as endorphins, enkephalins, dynorphins or endomorphins involved in mechanisms of endogenous control of pain transmission, naturally occurring amphibian opioid peptides became target molecules for scientific research. Dermorphins and deltorphins are opioid peptides isolated from skin of South American frogs [3,4]. They have the *N*-terminal amino acid sequence Tyr-D-Xaa-Phe, similar for the whole group, containing

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D-isomer of amino acid in the second position, which is essential for binding to opioid receptors and highly contributes to metabolic stability of these peptides [5,6]. Central application of dermorphin, a highly potent and selective MOR agonist, elicited analgesia which was 200 to about 2000 times stronger than that induced by MF, depending on the test used [7,8,9]. Antinociceptive effects of deltorphins, potent delta opioid receptor (DOR) agonists, are less pronounced [10,6,11] but activation of central DOR is known to be associated with reduced severity of adverse effects, such as respiratory depression [12], constipation [13,14] and physical dependence [15] common due to the treatment with MOR agonists.

Both dermorphins and deltorphins cross the blood–brain barrier and are active after peripheral administration [16,17]. Analgesia induced by systemic dermorphin is relatively long-lasting and still stronger than that induced by MF, although only a small fraction of this peptide enters the central nervous system [7,9]. Poor resistance to proteolytic degradation and low bioavailability are important factors limiting clinical use of peptide analgesics. Therefore, elaboration of novel opioid peptide analogs, active after systemic administration, would comprise an important source for new potent analgesic drugs with several advantages over nonpeptide agents like high activity and specificity, low toxicity and minimal interactions with other drugs.

Since the discovery of amphibian opioid peptides, numerous synthetic analogs were developed with diverse characteristics of opioid receptor affinity and potency, but relatively few exerted potent analgesia after peripheral administration. Early demonstration of antinociception stronger than MF was possible after sc administration of linear dermorphin tetrapeptide analogs with D-arginine substituted for D-alanine in the second position [18]. Since then, a number of highly potent derivatives of [D-Arg^2] 1-4 dermorphin and endogenous opioid peptides active in pain transmission after systemic application in animals and humans were developed [for review see 19,20].

Structure–activity studies demonstrated that conformational restriction of linear analogs of endogenous opioid peptides through incorporation of cyclic structural elements allowed for obtaining compounds with high affinity to opioid receptors and improved metabolic stability [21–33]. Among the cyclic analogs of natural opioid peptides restricted *via* a urea bridge, designed in recent years [22–26,32,33], two were tested *in vivo* but following only intracerebroventricular (icv) injection [34,35].

Recently, Bańkowski et al. [36] synthesized a series of *N*-substituted amides of cyclic ureidopeptides, mainly *N*-alkyl- and *N*-(ureidoethyl)amides of new dermorphin/deltorphin tetrapeptide analogs. All those compounds containing D-isomer of lysine or ornithine in the second position exhibited high resistance to enzymatic degradation. Preliminary studies of antinociceptive activity *in vivo* demonstrated that among these newly synthesized ureidopeptides, the highest activity was exhibited by D-Lys^2, Dab^4 *N*-(ureidoethyl)amide ({[H-Tyr-D-Lys(&^{1})-Phe-Dab(&^{2})-$CH_2CH_2NHCONH_2$][&^{1}CO&^{2}]} – cUP-1). Intravenous (*iv*) administration of cUP-1 resulted in significant, stronger than MF, antinociception in hot plate and tail-flick tests in mice.

The current studies were aimed at further evaluation of cUP-1 antinociceptive activity in animal models of somatic and visceral pain. Additionally, the influence of cUP-1 on spontaneous locomotion, motor coordination and muscle strength was estimated.

Materials and methods

Experiments were performed on male BALB/c mice weighing 20–25 g, except the paw pressure (Randall–Selitto) test where male Wistar rats weighing 180–240 g were used. The animals were purchased from the Centre of Experimental Medicine (Medical University of Białystok, Poland) and then housed in cages in a standard 12/12 light/dark cycle with light on from 7.30 am. Water and food were available *ad libitum* until the animals were transported to the laboratory approximately 1 h before experiments. All behavioral testing was performed between 9:00 am and 4:00 pm after one week of adaptation. Animal care and handling procedures were in accordance with the guidelines of the International Association for the Study of Pain (IASP) on the use of animals in pain research and the procedures of the study were approved by the IV Local *Ethics Committee for Animal* Experimentation in Warsaw.

cUP-1 was synthesized as described in detail in Ref. [36]. Both techniques were used for its synthesis: the solid phase adopted for the preparation of peptide *N*-(ureidoethyl)amides [37], as well as the classical synthesis in solution. The structure was confirmed by HR-MS spectra and NMR studies. RP-HPLC-purified cUP-1 (98.3% purity) was used in this study.

cUP-1 was dissolved in aqua pro injectione and administered in doses from 0.5 to 2 mg kg^{-1} (0.64–2.56 μmol kg^{-1}) in nociceptive tests and in doses of 1, 4 and 8 mg kg^{-1} (1.28, 5.12 and 10.24 μmol kg^{-1}) in the evaluation of locomotor activity in the open field test, motor coordination (rota-rod test) and the grip strength.

Morphine sulphate (Morphini sulfas WZF, WZF Polfa S.A, Poland) was used as a reference agent in nociceptive tests and administered in doses of 0.5, 1 and 2 mg kg^{-1} (1.49, 2.98 and 5.96 μmol kg^{-1}). The studied substances or vehicle were injected *iv* into the tail vein in a volume of 5 ml kg^{-1}.

Paw pressure test

The nociceptive threshold to mechanical stimulus was assessed according to the modified Randall–Selitto method [38,39]. Progressively increasing and digitally controlled mechanical force was applied to the middle portion of the dorsal surface of the rat's left hind paw with a hand held pressure applicator (IITC Life Science, USA). The pressure force eliciting a paw withdrawal reaction was automatically recorded in grams. Each trial was repeated three times at 1-min intervals. Rats were submitted to a single training session for acclimation to handling and the experiment conditions before the tests commenced. Rats were randomly assigned to five groups (n = 10) and paw withdrawal to mechanical stimulation was measured 24 h before (baseline) and 30 and 60 min after *iv* administration of cUP-1 or MF in doses of 1 and 2 mg kg^{-1} or vehicle in the control group.

Formalin test

A formalin test (FT) was used as a model of acute and tonic somatic pain [40]. A biphasic behavioral response was induced by the injection of 20 μl of 5% formalin solution into the dorsal surface of a mouse's right hind paw. Mice were placed into individual clear containers with angled mirrors underneath. After 30 min of acclimation, mice were injected with formalin solution and returned to the containers for observation. The total time spent on licking of the injected paw was recorded in the first (0–5 min) and the second (10–60 min) phase of FT. To determine the antinociceptive effect of the tested compounds, groups of mice (n = 8) were given *iv* injection with cUP-1 or MF in doses of 0.5, 1 and 2 mg kg^{-1} or vehicle 30 min before formalin application.

Writhing test

A writhing test was used as a model of visceral pain [41]. Mice (n = 10) were injected with 0.75% acetic acid *ip* in a volume of