Peptides 60 (2014) 102–106

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Anti-inflammatory and antinociceptive action of the dimeric enkephalin peptide biphalin in the mouse model of colitis: New potential treatment of abdominal pain associated with inflammatory bowel diseases

Marta Sobczak[a], Andrzej Pilarczyk[a], Mateusz Jonakowski[a], Agata Jarmuż[a], Maciej Sałaga[a], Andrzej W. Lipkowski[b], Jakub Fichna[a,b,*]

[a] Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, Lodz, Poland
[b] Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

ARTICLE INFO

Article history:
Received 26 June 2014
Received in revised form 11 August 2014
Accepted 11 August 2014
Available online 20 August 2014

Keywords:
Biphalin
Inflammatory bowel diseases
Inflammatory pain
Mixed opioid agonist
Opioid receptors

ABSTRACT

Biphalin, a mixed MOP/DOP agonist, displays a potent antinociceptive activity in numerous animal models of pain. The aim of the study was to characterize the anti-inflammatory and antinociceptive action of biphalin in the mouse models of colitis. The anti-inflammatory effect of biphalin (5 mg/kg, twice daily, i.c. and i.p.) was characterized in a semi-chronic mouse model of colitis, induced by i.c. injection of trinitrobenzenesulfonic acid (TNBS). The antinociceptive action of biphalin (5 mg/kg, i.p. and i.c.) in inflamed mice was assessed in mustard oil-induced model of visceral pain and in the hot plate test. In the semi-chronic mouse model of colitis, biphalin i.c. (5 mg/kg), but not i.p. improved colitis macroscopic score (2.88 ± 0.19 and 4.99 ± 0.80 units for biphalin and vehicle treated animals, respectively). Biphalin injected i.p. and i.c. (5 mg/kg) displayed a potent antinociceptive action in the mustard oil-induced pain test. In the hot plate test, biphalin (5 mg/kg, i.p.) produced a potent antinociceptive activity in inflamed mice, suggesting central site of action. Our data suggest that biphalin may become a novel opioid-based analgesic agent in IBD therapy and warrant further investigation of its pharmacological profile.

Introduction

For centuries, opioids have been used as potent analgesics for acute or chronic treatment to relieve moderate to severe pain, mainly in cancer patients or after major surgical interventions [11]. The most common and severe side effects of the chronic use of opioids include inhibition of gastrointestinal motility (i.e. opioid-induced bowel dysfunction and opioid-induced constipation), respiratory depression and physical dependence. However, opioids are still regarded as the gold standard in pain therapy [27]. The search for a strong analgesic agent without side effects similar to those occurring after morphine administration has been a longstanding interest in drug development.

Biphalin ((Tyr-D-Ala-Gly-Phe-NH-)$_2$), first synthesized by Lipkowski et al. [13], is a linear octapeptide with a dimeric structure based on two identical portions derived from enkephalins, joined tail to tail by a hydrazide bridge. Biphalin is a MOP (μ-opioid receptor) and DOP (δ-opioid receptor) agonist with high binding affinity (EC_{50} 1–5 nM) and potency. It was reported that biphalin exerts a high metabolic stability in the serum, with the metabolic half-life of 87 ± 1.8 min [10]. Moreover, biphalin is a 257- and 6.7-fold more potent analgesic than morphine and etorphine, respectively, in tail flick test and causes low physical dependence liability after chronic intravenous (i.v.) or intraperitoneal (i.p.) injections [29]. In addition, it has been evidenced that biphalin acts as an immunomodulator by stimulating human T cell proliferation, natural killer cell cytotoxicity in vitro and IL-2 production [16]. However, very little is known about the effect of biphalin in the gastrointestinal (GI) tract. There is only one report by Horan et al. [10] showing that biphalin injected intracerebroventricularly (i.c.v.) inhibited the GI transit in a dose-dependent manner (A_{50} value of 39.3 pmol/mouse).

IBD constitute a large group of chronic disorders of the gastrointestinal (GI) system [24]. The most common within this group

* Corresponding author at: Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, Mazowiecka 6/8, 92-215 Lodz, Poland.
Tel.: +48 42 272 57 07; fax: +48 42 272 56 94.
E-mail address: jakub.fichna@umed.lodz.pl (J. Fichna).

http://dx.doi.org/10.1016/j.peptides.2014.08.005
0196-9781/

are ulcerative colitis and Crohn's disease. The pathophysiology of IBD is still unknown and many factors, including genetic, microbial and environmental are believed to trigger the disease. The major IBD symptoms involve inflammation and damage of the intestinal mucosa (with periods of remissions) and abdominal pain. Diarrhea, fecal bleeding, weight loss and fatigue are also present. Currently available anti-IBD therapies allow to alleviate the symptoms and do not lead to complete cure, therefore novel potential drug candidates are searched [15].

The aim of the study was to characterize the anti-inflammatory action of biphalin in the mouse model of colitis. We also evaluated the antinociceptive potential of biphalin in inflammation.

Materials and methods

Animals

Male Balb/C mice, weighing 22–26 g, were used for the study. The animals were housed at a constant temperature (22 ± 1 °C) and maintained under a 12-h light/dark cycle in sawdust coated transparent cages with access to laboratory chow and tap water. The procedures used in this study were approved by the Local Ethical Committee for Animal Research with the following numbers: #534/2011.

Drugs

Biphalin was synthesized in Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland. Biphalin was dissolved in DMSO and further diluted with saline to the final DMSO concentration of 5%. In all experiments biphalin was injected at the dose 5 mg/kg (i.p. or i.c.); this dose was established based on data reported earlier. The solution of 5% DMSO in saline alone was used for control groups and had no influence on observed parameters.

TNBS model

Colitis was induced by i.c. instillation of trinitrobenzenesulfonic acid (TNBS) in 30% EtOH in saline [6]. In this semi-chronic colitis model, the therapeutic effect of biphalin was assessed as follows: inflammation was induced on day 1 and the animals received biphalin treatment (5 mg/kg, twice daily, i.p. or i.c.) between day 3 and day 7. On day 8, all mice were sacrificed and the macroscopic score was performed. Briefly, the colon was isolated, opened longitudinally and washed. The following parameters were assessed: area of inflammation and ulcers, adhesion, reddening, the presence of diarrhea and fecal blood, the length and thickness of the colon. The colon tissue samples were also taken for analysis of MPO activity, as described previously [25].

Determination of MPO activity

The increase in MPO activity is associated with infiltration of neutrophils into colonic tissue and indicates the degree of inflammation. To assess the MPO activity in this study, the sections of the distal colon (20–30 mg) were isolated, washed and homogenized in hexadecyltrimethylammonium bromide buffer (0.5% hexadecyltrimethylammonium bromide in 50 mM potassium phosphate buffer, pH 6.0; 50 mg of tissue/ml) using Ika Ultra Turrax Disperser (IKA, Germany). The homogenates were centrifuged (15 min, 13,200 × *g*, 4 °C), and the supernatants were transferred into new tubes. Seven microliters of supernatant was pipetted on a 96-well plate, and 200 μl of 50 mM potassium phosphate buffer (pH 6.0) containing 0.167 mg/ml O-dianisidine hydrochloride and 0.05 ml of 1% hydrogen peroxide was added to each well. The absorbance was measured at 450 nm for 0, 30 and 60 s (iMARK Microplate Reader; Bio-Rad, Hercules, CA, USA). All assays were performed in triplicates. The obtained results were expressed in milliunits per gram of wet tissue. A unit of MPO activity was defined as that converting 1 mmol of H_2O_2 to water in 1 min at room temperature.

Behavioral response to mustard oil

Mustard oil-induced pain responses

To assess the antinociceptive action of biphalin in mice with acute colitis, colonic inflammation was induced by instillation of TNBS on day 1, as described above. The development of colitis was monitored on days 1–4. On day 4, animals were divided into two groups: biphalin-treated and control. Biphalin or vehicle were injected i.p. or i.c. and 15 min later behavioral response to pain, induced by the intracolonic instillation of 1% mustard oil (MO, allyl isothiocyanate) in 70% EtOH in 0.9% NaCl), was measured. Briefly, mice were separated into clear plastic boxes (20 cm × 20 cm × 15 cm) and allowed a 5-min recovery after MO administration. Spontaneous behaviors were then observed and counted for 20 min. The behavioral pain responses included licking of the abdomen, stretching the abdomen, squashing of lower abdomen against the floor, and abdominal retraction and each behavioral pain response was counted as 1 [22].

Hot plate test

To assess the effect of biphalin on thermal pain in mice with acute colitis, colonic inflammation was induced by instillation of TNBS on day 1, as described above. The development of colitis was monitored on days 1–4. On day 4, animals were divided into two groups: biphalin-treated and control. The hot plate test was performed according to the method of Eddy and Leimbach [4]. A transparent plastic cylinder (14 cm diameter and 20 cm height) was used to confine the mouse on the heated surface of the plate. The plate was heated until 55 °C and the latencies to paw licking, rearing and jumping were measured. A cut-off time of 240 s was used to avoid tissue injury. The animals were placed on the hot plate 15 min after injection [7].

Statistics

The results are expressed as mean ± SEM. Statistical analysis was performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). ANOVA followed by Newman–Keuls post hoc testing was used. P values <0.05 were considered statistically significant.

Results and discussion

The structure of biphalin derives from enkephalins, short endogenous peptides synthesized mainly in the CNS and by gastric and intestinal endocrine cells in the GI system [3]. The crucial role of enkephalins is the inhibition of pain signaling in the CNS and in the periphery. Enkephalins are also formed in the immune cells and therefore may play a role in inflammation by suppression of formation of reactive oxygen and nitrogen species, as well as cytokines and enzymes that are involved in the immune defenses [28]. Recently, an increased level of Met-enkephalin was observed in colonic biopsies collected from inflamed area in IBD patients, compared to non-inflamed tissue obtained from the same patients [19].

Previously, the antinociceptive action of biphalin was assessed in the tail flick test after central (i.c.v. and i.t.) and peripheral (i.v. and s.c.) administration [10,12,14]. Interestingly, the effect of central biphalin was more potent in comparison with etorphine