Peptides 44 (2013) 105–110

Contents lists available at SciVerse ScienceDirect

Peptides

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

Synergistic analgesic effects between neuronostatin and morphine at the supraspinal level

Shao-Bin Yang[a], Ai-Min Yang[a], Ting-Ji Shao[b], Shu-Fang Su[a], Qiang Chen[a,*]

[a] Institute of Biochemistry and Molecular Biology, School of Life Sciences, Lanzhou University, 222 Tian Shui South Road, Lanzhou, 730000, PR China
[b] School of Pharmacy, Lanzhou University, 222 Tian Shui South Road, Lanzhou, 730000, PR China

ARTICLE INFO

Article history:
Received 23 December 2012
Received in revised form 10 March 2013
Accepted 11 March 2013
Available online 30 March 2013

Keywords:
Neuronostatin
Morphine
Opioid receptors
Synergistic analgesia
Mice

ABSTRACT

Neuronostatin, a 13-amino acid peptide, is encoded in the somatostatin pro-hormone. I.c.v. administration of neuronostatin produces a significant antinociceptive effect in the mouse tail-flick test, which is mediated by endogenous opioid receptor. However, the direct functional interaction between morphine and neuronostatin has not been characterized. In the present study, effect of neuronostatin on morphine analgesia was investigated in the tail-flick test. Our findings showed that i.c.v. administration of neuronostatin (0.3 nmol/mouse i.c.v.) significantly enhanced the antinociceptive effect of morphine (2.5, 5 or 10 μg/kg) at the supraspinal level. Results of antagonism experiments suggested that the synergistic analgesia induced by morphine and neuronostatin was mediated by μ- and κ-opioid receptors not δ-opioid receptor. In conclusion, there may be a cascade amplification phenomenon when morphine and neuronostatin were co-administered in acute pain model. The above results provide evidence for the potential use of neuronostatin in combination with morphine to control pain and addiction.

1. Introduction

Recently, a 13-amino acid peptide named neuronostatin (NST) has been discovered, which is encoded in the somatostatin pro-hormone. Neuronostatin is a brain/gut peptide, plays important physiological roles in the regulation of brain and other organs [20]. I.c.v. administration of neuronostatin leads to an increase in mean arterial pressure and a decrease in food and water intake, and these actions rely on the central melanocortin system [29,30]. Neuronostatin induces reduction in memory retention and the hippocampus is involved in the physiological effect [7]. The latest research suggested that neuronostatin activates p38 mitogenactivated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) in the heart regulating cardiac contractile function and cardiomyocyte survival [23,25]. In addition, i.c.v. injection of neuronostatin produces a dose and time-related anti-nociceptive effect in the tail-flick test and induces hyperalgesic effect in the formalin test in mice. These actions are dependent upon the melanocortin and opioid system [27,28].

Morphine with a potent analgesic property has been widely used for treatment of moderate to severe pain states. However, morphine is associated with a number of problematic side-effects, such as tolerance, dependence, constipation, addiction liability and opioid-induced hyperalgesia [19,21]. Multimodal analgesic techniques may produce analgesia at lower doses to decrease unwanted side effects [5,19]. It is reported that systemic morphine attenuates the analgesia by opioid-receptor [13,16,18]; other study showed that the endogenous opioid peptides may involve in the antinociceptive effect [14,16,21,22,26]. Recently we have reported that i.c.v. administration of neuronostatin produces antinociceptive effect in the tail-flick test, which depressed the nociceptive inputs at the spinal level by the brainstem decending inhibitory system [27]. Neuronostatin and morphine seem to have a functional interaction in the nociceptive response. However, to our knowledge, the direct functional interaction between morphine and neuronostatin has not been reported. In our present study, we intend to investigate the effect of the minimal analgesic dose of neuronostatin at the supraspinal level (i.c.v.) on morphine analgesia in the tail-flick test (the acute nociception model).

2. Materials and methods

2.1. Animals

Male Kunming mice, weighing 19–21 g, were supplied by the Animal Center of Lanzhou University (Lanzhou, China). The animals were housed (5–6/cage) at room temperature of 22–24 °C and 50–60% relative humidity with free access to laboratory chow and water. The animals were allowed to adapt to this environment for a period of 3–5 days before the experiments. All testing procedures

* Corresponding author. Tel.: +86 931 8915316; fax: +86 931 8915316.
E-mail address: chenq@lzu.edu.cn (Q. Chen).

0196-9781/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.peptides.2013.03.025

were approved of by the guidelines of the Ethics Committee of Animal Experiments of Lanzhou University.

2.2. Drugs

Neuronostatin (Leu-Arg-Gln-Phe-Leu-Gln-Lys-Ser-Leu-Ala-Ala-Ala-Ala-NH_2) was synthesized by manual solid-phase synthesis using standard Fmoc-chemistry as described in our previous report [27]. Naloxone hydrochloride dihydrate, β-funaltrexamine hydrochloride (β-FNA), naltrindole hydrochloride (NTI) and nor-binaltorphimine dihydrochloride (nor-BNI) were purchased from Sigma Chemical Company (USA). Morphine hydrochloride (10 mg/ml) was the product of Shenyang First Pharmaceutical Factory. All reagents were dissolved in normal saline (NS).

2.3. Intracerebroventricular injection

Intracerebroventricular (i.c.v.) administration was performed following the method described by Haley and McCormick [10]. The injection site was above the right lateral ventricle (1.5 mm from the middle, 1 mm from the bregma and 3 mm from the surface of the skull). Drugs were administered in a volume of 4 μl at a constant rate of 10 μl/min using a 10-μl Hamilton microsyringe. The proper injection site was verified in pilot experiments by administration and localization of methylene blue dye.

2.4. Tail immersion test

The nociceptive response was assessed with 49.0 °C warm water tail immersion assay. Every mouse was used only once. All experiments began at 10:00 a.m. and were performed. The animals were gently restrained by hand and the distal half of the tail was immersed in a constant temperature water bath set at 49.0 °C. The time elapsed prior to removal of the tail from the water surface was taken as the tail withdrawal latency (TWL). Every mouse was first tested for latency by immersing its tail in the water and recording the response time. Only those mice with the baseline latency within the range of 2.5–4.5 s were selected for further studies, and cut-off latency was set at 15 s to avoid damage to the tail. Control latencies were determined for by measuring the time required for the mouse to withdraw its tail from the water 20 min before drug treatment. A series of six sequential predrug administration latency measurements were made to establish a stable baseline, each with a 10 min interval. The latencies of the last four tests were averaged to provide a baseline value. Tail withdrawal time was determined immediately before 5, 10, 20, 30, 40, 50 and 60 min after injection. Typically, these values varied by <10%. The nociceptive effects in the tail immersion tests are calculated as the percentage change of tail withdrawal latency from the baseline level according to the formula: percentage change of TWL = 100 × [(post-drug latency − baseline latency)/baseline latency)] [24]. After experiment, mice were immediately sacrificed by cervical dislocation.

2.5. Experimental design

Our recent research reported that i.c.v. administration of neuronostatin produced a dose- and time-related antinociceptive effect in the tail-flick test in mice and the effect was significantly reversed by classical opioid receptor antagonist naloxone. In the present study, in order to further investigate the direct functional interaction between neuronostatin and morphine. The experiment was designed to two sections: (1) to examine the role of neuronostatin in mediating the effect of morphine analgesia, mice were co-administered (i.c.v.) with neuronostatin (0.3 nmol/mouse) and morphine (2.5, 5 and 10 μg/kg) [9,27]. In addition, morphine (2.5, 5 and 10 μg/kg) was injected alone to test the effect of morphine analgesia at each same dose. (2) In order to investigate the mechanisms on neuronostatin in the effect of morphine analgesia at the supraspinal level, classical opioid receptor antagonist naloxone (1 mg/kg), μ-, δ-, κ-opioid receptor antagonist β-FNA (10 nmol/mouse), NTI (10 nmol/mouse) or nor-BNI (10 nmol/mouse) were used. Naloxone was given subcutaneously (s.c.) 10 min prior to administration of neuronostatin and morphine, other antagonists were co-administered (i.c.v.) with neuronostatin (0.3 nmol/mouse) and morphine [9].

2.6. Statistical analysis

Data were presented as mean ± standard error of the mean (SEM). Statistical analyses of the data were performed by One-way analysis of variance (ANOVA) followed by the LSD post hoc test to assess the significance of differences among groups. Student's *t*-test was performed to test the difference between the two groups. In all statistical comparisons, differences with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of neuronostatin on the analgesia induced by morphine

The dose- and time-related analgesic effect of i.c.v. administration of morphine alone in 49.0 °C warm-water tail immersion assay in conscious mice is illustrated in Fig. 1A. Compared to the saline, i.c.v. administration of morphine (2.5, 5 or 10 μg/kg) produced an obviously dose-related increase in tail withdrawal latencies, reaching a maximal antinociceptive response at 10 min after injection and the effect continued about 60 min. The percent change of TWL at 10 min after i.c.v. administration of morphine (2.5, 5 or 10 μg/kg) was 39.70 ± 4.46%, 55.48 ± 4.66%, 89.95 ± 7.71%, respectively (vs. NS group, each $P < 0.001$). I.c.v. injection neuronostatin (0.3 nmol/mouse) produced a transient increase in tail withdrawal latencies, the change was 9.22 ± 3.59% at 10 min and rapidly returned to the baseline at 20 min. Compared to morphine (2.5, 5 or 10 μg/kg) alone, co-administration (i.c.v.) of morphine and neuronostatin (0.3 nmol/mouse) increased the extent of antinociception (Fig. 1B–D). As mentioned above, i.c.v. injection morphine (2.5 μg/kg) alone induced high antinociception level, and co-administration (i.c.v.) of morphine and neuronostatin, higher level of antinociception was observed at 10 min, the percentage change of TWL was 85.02 ± 5.64% (vs. neuronostatin, $P < 0.001$). The value gradually dropped to the baseline at 60 min (Fig. 1B). As shown in Fig. 1C, i.c.v. injection morphine (5 μg/kg) alone led to a maximal analgesic response at 10 min, similarly, the same concentration of morphine in combination with neuronostatin (0.3 nmol/mouse) produced an increase in analgesic potency at 10 min, and the percentage change of TWL was 103.85 ± 11.58% (vs. neuronostatin, $P < 0.001$). The value declined to the baseline at 60 min. As shown in Fig. 1D, a maximal analgesic response at 10 min was elicited by 10 μg/kg of morphine (i.c.v.) alone, at the same time, co-administration of morphine and neuronostatin resulted in an increase in analgesic potency, the percentage change of TWL was 122.09 ± 9.33% (vs. neuronostatin, $P < 0.001$), the maximal response dropped to the baseline at 60 min. As shown in Fig. 1E and F, the enhanced analgesia induced by co-injection of morphine (2.5 μg/kg) and neuronostatin was equivalent with the analgesia of morphine (10 μg/kg). Furthermore, morphine (5 μg/kg) and neuronostatin caused enhanced analgesia was much more than morphine (10 μg/kg) alone.