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Ghrelin receptor agonist, GHRP-2, produces antinociceptive effects at the supraspinal level via the opioid receptor in mice

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ABSTRACT

GHRP-2 is a synthetic agonist of ghrelin receptor. GHRP-2 has similar physiological functions with ghrelin. In our previous study, ghrelin (i.c.v.) could induce analgesic effect through an interaction with GHS-R1α and with the central opioid system in the acute pain in mice. To date, the function of GHRP-2 in pain processing was not understood. Therefore the aim of this study was to investigate the effects of GHRP-2 on pain modulation at supraspinal level in mice using the tail immersion test. Intracerebroventricular (i.c.v.) administration of GHRP-2 (0.1, 0.3, 1, 3 and 10 nmol/L) produced a concentration- and time-related antinociceptive effect. This effect could be fully antagonized by GHS-R1α antagonist [D-Lys^3]-GHRP-6, indicating that the analgesic effect induced by GHRP-2 is mediated through the activation of GHS-R1α. Interestingly, naloxone, naltrindole and nor-binaltorphimine, but not β-funaltrexamine, could also block the analgesic effect markedly, suggesting that δ- and κ-opioid receptor is involved in the analgesic response evoked by GHRP-2. Moreover, i.c.v. administration of GHRP-2 potentiated the analgesic effect induced by morphine (i.c.v., 1 nmol/L) and this potentiated effect could not be reversed by [D-Lys^3]-GHRP-6. Thus these findings may be a new strategy on investigating the interaction between ghrelin system and opioids on pain modulation. Furthermore, GHRP-2 may be a promising peptide for developing new analgesic drugs.

1. Introduction

Pain perception is a complex and externally inaccessible experience determined by the integration of complex processes modulated by central factors, including neurotransmitters and neurohormones [10]. The management of pain is considered to be a major clinical problem [2]. Opioids have been used for treating moderate to severe pain, but treatment with these drugs leads to the induction of side effects such as analgesic tolerance, physical dependence and drowsiness, etc. Therefore, the finding of better analgesic drugs that have analgesic property without hazardous side effects is essential skills for pain management.

Ghrelin, a 28-amino-acid peptide, was discovered from rat gastric endocrine cells in 1999 [12]. Ghrelin is the endogenous ligand for growth hormone secretagogue receptor 1 alpha (GHS-R1α) which is G protein coupled receptor in the cell membrane [3,12]. The selective antagonist of GHS-R1α is [D-Lys^3]-GHRP-6 [20]. Ghrelin exerts many physiological and pathological functions through GHS-R1α. Ghrelin plays roles of food intake [33], stimulation of growth hormone (GH), ACTH, cortisol, prolactin (PRL) secretion [23,25], regulation of cardiovascular actions [15], treatment of cancer [24], and modulation of reproduction, etc. [1,21]. Previous studies have shown that ghrelin inhibits the inflammatory pain in rats through an interaction with the central opioid system [26] and a ghrelin receptor different from GHS-R1α [27]. Other researchers have revealed that ghrelin attenuates chronic neuropathic pain [7,13] and prevents cisplatin-induced mechanical hyperalgesia and cachexia, etc. [5]. In our previous study, i.c.v. administration of ghrelin could induce analgesic effect through an interaction with GHS-R1α and with the central opioid system in the acute pain in mice [32].

Before ghrelin was isolated, several synthetic peptides described as growth hormone secretagogues were developed as ligands

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for GHS-R1α [18,28]. One of these peptides, termed growth hormone-releasing peptide-2 (GHRP-2, D-Ala-D-β-Nal-Ala-Trp-Phe-Lys-NH_2), was found to be a full agonist at the GHS-R1α [18,28]. Like ghrelin, GHRP-2 stimulates the release of GH [11], and exertes GH-independent effects such as stimulation of food intake [14], induction of adiposity [30]. In addition, ghrelin and GHRP-2 exhibit anti-inflammatory effects in both in vitro and in vivo models [6,27]. In our previous study, i.c.v. administration of ghrelin and GHRP-2 could inhibit the analgesic effect induced by intraperitoneal (i.p.) administration of morphine [34]. These research studies have shown that ghrelin and GHRP-2 have some interaction with opioid system. However, ghrelin has a short half-life, and hence its clinical utility may be limited [22,29]. In this regard, GHRP-2 is a lower molecular weight compound with a longer half-life than ghrelin [28]. Furthermore, there is no research about the effects of GHRP-2 on the acute heat pain and its mechanism(s). Therefore, we have now investigated the effects of GHRP-2 on the acute heat pain and its mechanism(s). Thus, the present study aimed to: (1) evaluate the effects of i.c.v. administration of GHRP-2 on pain modulation and morphine-induced analgesia, and (2) investigate the mechanisms involved in the effect.

2. Materials and methods

2.1. Animals

Male Kunming strain mice (18–22 g) were used in all experiment. Use of the animals was reviewed and approved by the Animal Care and Use Committee of Medical College of Nanchang University. All animals were well cared and experiments were carried out according to the European Community guidelines for the use of experimental animals (86/609/EEC). Animals were housed in an animal room which was maintained at 22 ± 2 °C with a 12-h light, 12-h dark cycle (light on 8:30 a.m. to 8:30 p.m.) and 50–60% relative humidity. The animals were allowed to adapt to this environment for a period of 3 days before the experiments. Food and water were available ad libitum. Every effort was made to minimize the numbers and any suffering of the animals used in the following experiments. All the protocols in this study were approved by the Ethics Committee of Nanchang University, China.

2.2. Peptides and compounds

GHRP-2 [D-Ala-D-β-Nal-Ala-Trp-D-Phe-Lys-NH_2] were purchased from Chengdu Capgemini Biological Medicine Technology Development Co., Ltd, China. [D-Lys^3]-GHRP-6 (His-D-Trp-D-Phe-Lys-NH_2) was purchased from Phoenis Pharmaceuticals, Inc. Morphine hydrochloride was the product of Shenyang First Pharmaceutical Factory, China.

Naloxone hydrochloride dihydrate (Fluka, Beijing China), β-funaltrexamine hydrochloride (β-FNA, Sigma), naltrindole hydrochloride (NTI, Sigma) and nor-binaltorphimine dihydrochloride (nor-BNI, Sigma) are opioid receptor (OR) antagonists. Preparation of all stock solutions (except β-FNA) and their subsequent dilutions were performed in normal saline. Stock solutions were stored frozen in aliquots. The aliquots were thawed and used on the day of the experiment. The β-FNA aqueous solution was used promptly because of its instability.

2.3. Administration of drugs

Intracerebroventricular (i.c.v.) administration was performed following the method described by Haley and McCormick [9]. The injection site was 1.5 mm posterior and 1.0 mm lateral to the bregma, and 3 mm from the surface of the skull. Drugs were i.c.v. administered in a volume of 3 μl at a constant rate of 10 μl/min by using a 25 μl microsyringe. The proper injection site was verified in pilot experiments by administration and localization of methylene blue dye.

2.4. Tail withdrawal test

The tail withdrawal test was used to evaluate the acute nociceptive effects of the drugs. All experiments began at 10:00 a.m. and were performed following the method described by Ping Zeng et al. [34]. Every mouse was used only once. The animals were gently restrained by hand and the distal half of the tail was immersed in water at 48 ± 0.5 °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 3–5 s were selected for further studies, and a cut-off latency was set at 15 s to avoid damage to the tail. Before each drug trial, a series of six sequential pre-drug 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 control value. Typically, these values varied by <10%. Post-drug latency measurements were performed at 5, 10, 20, 30, 40, 50 and 60 min.

To investigate the participation of the GHS-R1α on the antinociceptive effect of GHRP-2 (i.c.v.), the specific ghrelin receptor antagonist [D-Lys^3]-GHRP-6 was co-injected with GHRP-2. In order to further investigate the nociceptive mechanisms elicited by i.c.v. administration of GHRP-2, classical opioid receptor antagonist naloxone was co-administrated with GHRP-2. To determine the type of opioid receptor involved in the antinociceptive effect of GHRP-2, β-funaltrexamine hydrochloride (β-FNA), naltrindole hydrochloride (NTI) and nor-binaltorphimine dihydrochloride (nor-BNI), μ-, δ-, κ-opioid receptor antagonists, respectively, were used.

2.5. Histology and statistical analysis

After completion of behavioral testing, mice were injected with methylene blue dye (3 μl), which was allowed to diffuse for 10 min. Then mice were decapitated, and their brains were removed and frozen. Gross dissection of the brains was used to verify the proper injection site. Only the data from those animals with dispersion of the dye throughout the ventricles were used in the study. The nociceptive effects in the above tests were calculated as the percentage change of tail withdrawal latency (TWL) from the baseline level according to the formula: percentage change of TWL = [(postdrug latency − predrug latency)/predrug latency] × 100. The raw data from each animal were converted to area under the curve (AUC). The AUC data over the period 0–60 min were calculated and used for statistical analysis. The result is presented as mean value ± standard error of the mean (SEM). Each group consisted of 8–12 mice. Significant differences between groups were determined by means of one-way ANOVA followed by the Bonferroni test. In all statistical comparisons, $P < 0.05$ was used as the criterion for statistical significance.

3. Results

3.1. The effects of GHRP-2 on the nociception after i.c.v. administration

Fig. 1 illustrated the concentration- and time-related analgesic effect of i.c.v. administration of GHRP-2 in 48.5 °C warm-water tail immersion test in conscious mice. The i.c.v. administration