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Research paper

Antidepressant-like effects exerted by the intranasal administration of a glucagon-like peptide-2 derivative containing cell-penetrating peptides and a penetration-accelerating sequence in mice

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ABSTRACT

The intracerebroventicular (i.c.v.) administration of glucagon-like peptide-2 (GLP-2) to rodents was shown to have antidepressant-like effects in imipramine-resistant depression-model mice. In order to utilize GLP-2 as a clinical treatment tool for depression, we herein focused on the intranasal delivery that is non-invasive approach, because the i.c.v. administration is invasive and impractical. In the present study, we prepared a GLP-2 derivative containing cell penetrating peptides (CPPs) and a penetration accelerating sequence (PAS) (PAS-CPPs-GLP-2) for the intranasal (i.n.) administration. PAS-CPPs-GLP-2 (i.n.) exhibited antidepressant-like effects in the forced-swim test (FST) and tail suspension test (TST) in naïve mice as well as adrenocorticotropic hormone (ACTH) treated-mice. However, PAS-CPPs-GLP-2 (i.v.) and the GLP-2 derivative containing CPPs without a PAS (CPPs-GLP-2) (i.n.) did not affect the immobility time in the mouse FST. Moreover, fluorescein isothiocyanate (FITC)-labeled PAS-CPPs-GLP-2 (i.n.), but not FITC-labeled CPPs-GLP-2 (i.n.) was distributed through the mouse brain after the FST session. These results suggest that PAS-CPPs-GLP-2 is effective for i.n. delivery to the brain, and may be useful in the clinical treatment of major depression.

1. Introduction

Proglucagon-derived glucagon-like peptide-2 (GLP-2) is a 33-amino acid peptide produced in the gut and central nervous system (CNS) [1,2]. GLP-2 acts by binding to G protein-coupled GLP-2 receptors (GLP-2R) [3]. The GLP-2R gene is expressed not only in distinct gastrointestinal cells [3,4], but also in specific regions of the CNS including the dorsomedial hypothalamic nucleus (DMH), amygdala, thalamus, cerebellum, hippocampus, and cerebral cortex [5,6]. The intracerebroventricular (i.c.v.) administration of GLP-2 to rodents was previously reported to suppress food intake [7] and decrease blood pressure [8]. We also reported that the i.c.v. administration of GLP-2 exerted antidepressant-like effects in naïve mice [9] and adrenocorticotropic hormone (ACTH)-treated mice [10] through the modulation of the hypothalamic-pituitary-adrenal (HPA)-axis and the restoration of neurogenesis in the subgranular zone (SGZ) of the hippocampal dentate gyrus [11] in addition to activation of monoamine receptors and the changes in monoamine levels in the brain [9,10]. These findings raise the possibility that GLP-2 may become a novel treatment tool for major depression with different mechanisms of action from existing drugs. However,

Abbreviations: ACTH, adrenocorticotropic hormone; BBB, blood-brain barrier; BLA, basolateral amygdala; CNS, central nervous system; CPPs, cell penetrating peptides; DMH, dorsomedial hypothalamic nucleus; DMSO, dimethylsulfoxide; DPP-IV, dipeptidyl peptidase IV; FAM, carboxyfluorescein; FITC, fluorescein isothiocyanate; FST, forced-swim test; Fos, *c-fos* protein; GLP-2, glucagon-like peptide-2; HPA, hypothalamic-pituitary-adrenal; i.c.v., intracerebroventricular; IL, infralimbic cortex; i.n., intranasal; OB, olfactory bulb; OFT, open-field test; PAS, penetration accelerating sequence; Pr5, principal sensory trigeminal nucleus; PVH, paraventricular hypothalamic nucleus; RVLM, rostral ventrolateral medulla; SGZ, subgranular zone; TST, tail suspension test.

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the i.c.v. administration is invasive, costly, and impractical for the delivery of drugs into human brains, and thus we need to develop a non-invasive and effective way to deliver GLP-2 in the brain.

A difficulty associated with the development of peptides as CNS therapeutic agents is their limited ability to cross the blood-brain barrier (BBB) in order to reach their targets in the CNS at the pharmacological level following their systemic administration. Intranasal (i.n.) delivery offers a non-invasive alternative for getting protein and peptide drugs into the brain by utilizing olfactory neuronal distribution pathway in the cribriform plate, which may lead to direct nose-to-brain drug distribution [12].

Cell-penetrating peptides (CPPs), including arginine-rich peptides, have been shown to deliver various bioactive molecules with low membrane permeability into cells in order to regulate cell functions [13–15]. Macropinocytosis also plays an important role in the cellular uptake of arginine-rich CPPs, resulting in highly efficient intracellular delivery [16,17]. Macropinocytosis is transient, actin-driven fluid-phase endocytosis that involves membrane ruffling and the formation of large vacuoles called macropinosomes [18,19]. The addition of a penetration-accelerating sequence (PAS) to CPPs has been reported to enhance the efficiency of the intracellular delivery of bioactive peptides by promoting endosomal escape [20]. Although some CPP-peptide chimeras have reached clinical trials for the alleviation of pain, hearing loss, or stroke [21], it currently remains unknown whether the addition of a PAS to CPPs increases the effectiveness of peptide medicines.

In the present study, we synthesized several GLP-2 derivatives containing CPPs with or without a PAS, and examined antidepressant-like effects after the i.n. administration and distribution patterns in the CNS.

2. Materials and methods

2.1. Preparation of GLP-2 derivatives

PAS-CPPs-GLP-2 (FFLIPKG-RRRRRRRR-GG-HADGSFSDEMNTILDNLA ARDFINWLIQTKITD) (JPN Patent pending: No. 2014-184436, PCT applied for: WO 2016/035820 A1), CPPs-GLP-2 (RRRRRRRR-GG-HADGSFSDEMNTILDNLAARDFINWLIQTKITD), fluorescein isothiocyanate (FITC)-labeled PAS-CPPs-GLP-2, and FITC-labeled CPPs-GLP-2 were synthesized by the SCRUM Inc (Tokyo, Japan) with a peptide synthesizer (433A: Applied Biosystems) following the standard 9-fluorenylmethoxycarbonyl (Fmoc) method.

2.2. Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee at Tokyo University of Science, and were conducted according to the guidelines of the National Institute of Health and Japan Neuroscience Society. We used 5- to 7-week-old male ddY mice (Japan SLC, Shizuoka, Japan), and attempted to minimize the number of animals used and their suffering. A total of 118 ddY mice were used in the experiments. All animals were kept in a controlled environment, with a 12:12 h light schedule, temperature (23 °C), and relative humidity (55 ± 5%) for at least 5 days before the experiments, and were provided *ad libitum* access to food and water.

ACTH (Cortrosyn-Z: Daiichi-Sankyo, Tokyo, Japan) was diluted with saline. ACTH (0.45 mg/kg, s.c.) was administered once a day for 14 days. The dose of ACTH was based on previous studies [10,11]. Imipramine (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in saline and administrated for 6 days (30 mg/kg, i.p.).

2.3. The administration of GLP-2 derivatives

GLP-2 derivatives were dissolved in 16% dimethylsulfoxide (DMSO) (Wako Pure Chemical Industries, Osaka, Japan). Mice were anesthetized using isoflurane, and intranasally administered a GLP-2 derivative (a total amount of 3.6 μg/mouse) or vehicle (16% DMSO) (a total volume of 4 μl/mouse) via a micropipette in each nostril 30 min before the behavioral test. Nose drops administered to animals lying on their backs resulted in consistent deposition in the olfactory or respiratory epithelium. According to our previous studies [9,10], we administered the GLP-2 derivative for 2 days in naïve mice, or for 6 days in ACTH-treated mice.

2.4. Behavioral measurements

2.4.1. Tail suspension test (TST)

We performed the TST in order to examine the antidepressant-like effects of PAS-CPPs-GLP-2. The TST was performed using modifications to the procedures described in our previous study [9]. PAS-CPPs-GLP-2 or vehicle was administrated 30 min before the TST session once a day for 2 days (Fig. 1A). Mice were individually suspended by the tail from a horizontal ring (distance from floor, 27 cm) in a gray acrylic box (30 × 15 × 15 cm) (Bio Research Center, Nagoya, Japan) using adhesive tape affixed 2 cm from the tip of the tail. A 5-min test session was employed under bright (fluorescent room light) conditions, and was recorded through a web-camera system on a hard disk. The behavioral parameter measured was the time of immobility, which was defined as the time when mice were judged to cease escape-motivated behaviors.

2.4.2. Forced-swim test (FST)

We performed the FST in order to examine the antidepressant-like effects of the GLP-2 derivative. The FST was performed using modifications to the procedures described in our previous studies [9–11]. The FST was performed by placing a mouse in an acrylic cylinder (50 cm tall, 18 cm in diameter) containing a 7-cm water column (25 ± 1 °C). The water was replaced between every trial. Two swimming sessions were conducted on Day −1 and −2 (Fig. 1A): an initial 15-min pretest, followed by a 6-min test 24 h later. The GLP-2 derivative or vehicle was administrated 30 min before the FST session once a day for 2 days. Test sessions were recorded through a web-camera system on a hard disk in order to measure the time of immobility, with immobility being defined as floating passively in the water and only making slight movements to keep the head above the water line. The scored immobility time was blindly checked by the co-authors.

2.4.3. Open-field test (OFT)

We performed the OFT in order to examine the effects of PAS-CPPs-GLP-2 on locomotion activity and emotional responses. The OFT was performed using modifications to the procedures described in our previous study [22]. PAS-CPPs-GLP-2 or vehicle was administrated 30 min before the OFT session once a day for 2 days. The open-field apparatus consisted of a square area (40 × 40 cm) with 25-cm-high opaque walls. The floor was divided into 16 equal squares by lines. The test sessions were recorded through a web-camera system on a hard disk. The number of line crossings in the whole area was then counted for 5 min. The apparatus was wiped down with paper after the removal of each animal.

2.5. Distribution of the intranasally administrated GLP-2 derivative in the mouse brain

Mice were perfused transcardially with 0.1 M phosphate buffer (PB) (pH 7.4), followed by 50–100 ml of 4% (w/v) paraformaldehyde after the FST session. The brains were removed and postfixed at 4 °C