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Glucagon-like peptide-2-induced memory improvement and anxiolytic effects in mice

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ABSTRACT

We investigated the effectiveness of glucagon-like peptide-2 (GLP-2) on memory impairment in lipopolysaccharide (LPS)-treated mice, and anxiety-like behavior in adrenocorticotropic hormone (ACTH)-treated mice. In the Y-maze test, LPS (10 μg/mouse, i.c.v.) significantly decreased spontaneous alternation, which was prevented by pretreatment with GLP-2 (0.01–0.3 μg/mouse, i.c.v.). The GLP-2 treatment just before the Y-maze test also improved LPS-induced memory impairment. Continuous treatment with GLP-2 (3 μg/mouse, i.c.v.) had no effect on the open-field test in saline-treated or ACTH-treated mice. Chronic ACTH treatment did not cause anxiogenic effects in the elevated plus-maze test. GLP-2 showed weak anxiolytic-like effects in the elevated plus-maze test in ACTH-treated, but not saline-treated mice. Moreover, GLP-2 increased 5-HT, but not 5-HIAA and tryptophan hydroxylase 2 levels in the amygdala of ACTH-treated mice. Pharmacological depletion of 5-HT prevented the anxiolytic effects of GLP-2. These results suggest that GLP-2 protected and improved memory function in LPS-treated mice, and also had anxiolytic effects due to changes in the 5-HT system.

1. Introduction

Glucagon-like peptide-2 (GLP-2) is derived from a proglucagon precursor and is liberated via tissue-specific post-translational processing in the gut and central nervous system (Dhanvantari et al., 1996). GLP-2 was identified as a potent intestinotrophic hormone in rodents (Estall and Drucker, 2006), and enhanced nutrient absorption in rodents and patients with short bowel syndrome (Estall and Drucker, 2006; Wallis et al., 2009). On the other hand, an intracerebroventricular (i.c.v.) injection of GLP-2 inhibited food intake in rats (Tang-Christensen et al., 2000). In addition to its anorexigenic effects, GLP-2 protected hippocampal neurons from glutamate excitotoxicity (Lovshin et al., 2004), stimulated the proliferation of cultured rat cortical astrocytes (Velazquez et al., 2003), and induced hypotension (Sasaki-Hamada et al., 2012).

We previously reported that GLP-2 had antidepressant-like effects in the forced-swim test and tail suspension test (Iwai et al., 2009a). Decreasing immobility in these behavioral paradigms is thought to reflect its effects on the negative mood, which represents a kind of hopelessness, although the link between rodent performance in these tests and subjective human symptoms in depression requires further studies. Major depression was often accompanied by anxiety and memory impairment (Basso et al., 2007; Castaneda et al., 2008). These can also damage a patient's quality of life.

In the present study, we investigated the effects of GLP-2 on memory functions and anxiety behavior. In our previous report, GLP-2 had no effect on memory function in normal mice (Iwai et al., 2009a). We thus used memory-impaired mice, which were injected intracerebroventricularly (i.c.v.) with lipopolysaccharide (LPS), an endotoxin from Gram-negative bacteria and widely used in neuroinflammation studies (Iwai et al., 2008; Leon et al., 2008; Miwa et al., 2011). The effects of GLP-2 on anxiety have not yet been reported. In our preliminary findings, acute treatment with GLP-2 had no anxiolytic effects on rats. We thus continuously administered GLP-2 in chronically saline-treated or adrenocorticotropic hormone (ACTH)-treated mice at a dose that expressed antidepressant-like effects. ACTH-treated mice showed an increase in serum corticosterone levels before and after stress loading, which indicates overactivation of the hypothalamus–pituitary–adrenocortical (HPA) axis

Abbreviations: ACTH, adrenocorticotropic hormone; DRN, dorsal raphe nucleus; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; HPA, hypothalamic-adrenal axis; GLP-2, glucagon-like peptide-2; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; PCPA, p-chlorophenylalanine; TPH, tryptophan hydroxylase.

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(Iwai et al., 2013). Since chronic activation of the HPA axis caused anxiogenic responses in mice (Dedic et al., 2012), we hypothesized that ACTH-treated mice may have anxiogenic responses.

2. Materials and methods

All experimental protocols were approved by the Institutional Animal Care and Use Committee at Tokyo University of Science and Kitasato University, and conducted according to the guidelines of the National Institutes of Health and the Japan Neuroscience Society.

2.1. Animals

Six-week-old male ddY mice (Japan SLC, Shizuoka, Japan) were kept in a controlled environment, with controlled lighting (12 h light/dark cycle, lights on from 08:00 to 20:00), temperature (23 ± 1 °C), and relative humidity (55 ± 5%) for at least 5 days before the experiments, and were given free access to food and water.

2.2. Spontaneous alternation behavior in LPS-treated mice

2.2.1. Drug treatment

GLP-2 was administered 15 min before the LPS treatment or 30 min before the Y-maze test. LPS and GLP-2 were administered i.c.v. according to the method of Haley and McCormick (1957) in a volume of 5 μl/mouse under brief ether anesthesia. The dose of GLP-2 (0.01–0.3 μg/mouse for learning behavior, 3 μg/mouse for anxiety behavior) was determined based on our previous studies (Iwai et al., 2009a, 2009b, 2013; Sasaki-Hamada et al., 2012).

2.2.2. Y-maze test

Y-maze test sessions were carried out 3 days after the LPS treatment as described previously (Iwai et al., 2008, 2009b). Immediate working memory performance was assessed by recording spontaneous alternation behavior during a single session in the Y-maze, which was based on the likelihood of rodents entering an arm of the Y-maze that was not explored in the last two choices (Oka et al., 2000). Each mouse, new to the maze, was placed at the end of one arm and was allowed to move freely through the maze during an 8-min session. The series of arm entries were recorded visually. Alternation was defined as successive entries into the three arms, on overlapping triplet sets. The effect was calculated as percent alternation according to the following formula: percent alternation = {(number of alternations)/(total number of arm entries – 2)} × 100 (%).

2.3. Anxiety-related behavior in saline- or ACTH-treated mice

2.3.1. Drug treatment

ACTH (Cortrosyn-Z: Daiichi Seiyaku, Tokyo, Japan) was diluted with saline. ACTH (0.45 mg/kg, s.c.) or saline (vehicle control) was administered once a day for 14 days at 09:00–11:30. GLP-2 (Peptide Inc., Osaka, Japan) was dissolved in 0.01 M PBS. GLP-2 or PBS (vehicle control) was administered into the lateral ventricular (i.c.v.) region of the mouse brain once a day from Day-11 to Day-18, since no report has demonstrated the ability of GLP-2 to penetrate the blood–brain barrier. The i.c.v. administration (a volume of 5 μl/mouse) was performed under brief ether anesthesia according to the method described above. On the day of the open-field test (Day-17) or elevated plus-maze test (Day-18), GLP-2 or vehicle (saline or PBS) was administered 30 min before the test. p-Chlorophenylalanine (PCPA; 150 mg/kg, i.p.; WAKO, Osaka, Japan), an inhibitor of 5-HT synthase (tryptophan hydroxylase: TPH), or saline was administered from Day-14 to Day-17.

2.3.2. Open-field test

The open-field test was performed using a modification of the procedure described in our previous report (Sugiyama et al., 2012) under bright (fluorescent room light) conditions. The open-field apparatus consisted of a square area (40 × 40 cm) with opaque walls 25 cm high. The floor was divided by lines into 16 equal squares. Mice were placed in a corner of the open-field facing the opaque walls. The time spent in the center area (20 × 20 cm), latency to the center area, and the number of line crossings was then observed for 5 min on a monitor through a video camera system. The apparatus was cleaned after the removal of each animal.

2.3.3. Elevated plus-maze test

The elevated plus-maze test was performed on the day after the open-field test, using a modification of the procedure described in our previous report (Sugiyama et al., 2012) under bright (fluorescent room light, Fig. 4) or dark (20–30 Lux, Fig. 5) conditions. The elevated plus-maze apparatus was made of acrylic board and consisted of four arms set in a cross pattern from a neutral central square. Two opposite arms were delimited by vertical walls (closed arms, 30 × 5 × 15 cm), whereas the two other opposite arms had unprotected edges (open arms, 30 × 5 cm). The maze was elevated 50 cm above the ground. Animals were allowed at least 2 h for adaptation to the new environment before the experiments. All mice used for the elevated plus-maze tests were tested only once. At the beginning of the 5 min test session, each mouse was placed in the central neutral zone, facing one of the open arms. The total number of visits to the closed and open arms and the cumulative time spent (% time spent in open-arms) and visits (% entries in open-arms) in the open-arms were then observed on a monitor through a video camera system. An arm visit was recorded when the mouse moved half of its body into the arm. The apparatus was cleaned after the removal of each animal.

2.4. Analysis of the concentration of 5-HT and its metabolite with HPLC

The concentrations of 5-HIAA were determined by high-performance liquid chromatography (HPLC). Immediately after the treatment and the forced-swim test (Iwai et al., 2013) on Day 17, all ACTH-treated mice were killed by decapitation. The amygdala samples were quickly dissected. These samples were stored at –80 °C until further use. The tissues were homogenized in 300 μL of 0.2 M perchloric acid containing 100 μM EDTA (2 Na) and 100 ng of isoproterenol as an internal standard. To remove proteins completely, the homogenates were placed in cold water for 30 min and then centrifuged at 14,500 × *g* for 15 min at 0 °C; the upper layer was maintained at pH 3.0 using 1 M sodium acetate. Samples of 20 μL were analyzed by HPLC using electrochemical detection. The electrochemical detector (ECD-300, Eicom Co., Kyoto, Japan) was equipped with a graphite electrode (WE-3G, Eicom Co.) that was used at a voltage setting of 750 mV vs. an Ag/AgCl reference electrode. The mobile phase consisted of a 0.1 M sodium acetate/0.1 M citric acid buffer (pH 3.5) containing 17% methanol, 210 mg/L sodium 1-octanesulfonate, and 5 mg/L EDTA (2 Na). The monoamines were separated on a C-18 column (150 mm × 3.0 mm reversed-phase, EICOMPAK SC-5ODS, Eicom Co.). The mobile phase flow rate was maintained at 0.5 mL/min with a column temperature of 25 °C.

2.5. SDS-PAGE and western blotting

Amygdala tissue samples from ACTH-treated mice on Day 18 (immediately after the elevated plus-maze test) were suspended in lysis buffer [50 mM Tris (pH 7.5), 150 mM NaCl, 2% Triton X-100, 0.25%, v/v, and protease inhibitor cocktail (Nakarai Tesque, Tokyo, Japan)].