Behavioural Brain Research 204 (2009) 235–240

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Antidepressant-like effects of glucagon-like peptide-2 in mice occur via monoamine pathways

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ARTICLE INFO

Article history:
Received 26 April 2008
Received in revised form 4 June 2009
Accepted 9 June 2009
Available online 17 June 2009

Keywords:
Glucagon-like peptide-2
Forced-swim test
Antidepressant
Major depression
Serotonin
Noradrenaline
Dopamine

ABSTRACT

In this study, we investigated whether glucagon-like peptide-2 (GLP-2) had antidepressant-like effects in mice, and whether these activities were associated with monoamine systems in mice. Antidepressant-like effects were evaluated based on the immobility time in the forced-swim test. GLP-2 (1.5–6 μg/mouse, i.c.v.) significantly reduced the immobility time in a dose-dependent manner without affecting locomotor activity in the wheel running test and memory function in the step-down passive avoidance test. These effects were inhibited by pretreatment with metergoline (an antagonist of non-specific 5-HT receptors), parachlorophenylalanine (an inhibitor of 5-HT synthase), NAN-190 (an antagonist of 5-HT_{1A} receptors), yohimbine hydrochloride (an antagonist of α_2 adrenoceptors), atenolol (an antagonist of β_1 receptors), and raclopride (an antagonist of D_2 receptors), but not prazosin (an antagonist of α_1 adrenoceptors), ICI118551 (an antagonist of β_2 adrenoceptors), and SCH23394 (an antagonist of D_1 receptors). These results suggest that GLP-2 exerts antidepressant-like effects in the forced-swim test in mice, which are associated with 5-HT_{1A}, α_2, β_1 and D_2 receptors.

1. Introduction

Glucagon-like peptide-2 (GLP-2) is derived from a proglucagon precursor and liberated via tissue-specific post-translational processing in the gut and central nervous system [18,34]. GLP-2 was identified as a potent intestinotrophic hormone in rodents [19], and enhanced nutrient absorption in rodents and in human patients with short bowel syndrome [21,27]. On the other hand, intracerebroventricular (i.c.v) injection of GLP-2 inhibited food intake in rats [46]. In addition to the anorexigenic effects, GLP-2 protected hippocampal neurons from glutamate excitotoxicity [30], and stimulated the proliferation of cultured cortical astrocytes of rats [48].

GLP-2 receptor mRNA expression occurs in various brain regions including the dorsomedial hypothalamic nuclei (DMH), the nucleus solitary tract (NTS), the amygdala, the thalamus, the cerebellum, the hippocampus, and the cerebral cortex [29,30]. GLP-2 activated c-fos expression in DMH [46] and NTS [37], and increased cAMP concentration in hippocampal neurons, the brainstem and the cerebral cortex [30], indicating that GLP-2 activates at least the hippocampus, the cerebral cortex, DMH and NTS. These regions are implicated in the pathology of major depression, stress responses and/or actions of antidepressant treatment. Recent imaging studies in humans revealed that the hippocampus underwent selective volume reduction in stress-related neuropsychiatric disorders such as recurrent depressive illness [8,44]. In adult rats, chronic antidepressant treatment increased neurogenesis in the hippocampus [31], and upregulated the cAMP signaling pathway-mediated gene transcription in the cortex and the hippocampus [47]. Also, chronic vagus nerve stimulation that is used as the treatment of depression induces Fos expression in NTS. Despite of these evidences, the effects of GLP-2 on major depression have not been studied.

Major depression is a psychiatric disease that results in dramatic alterations in emotional, neurovegetative, and cognitive processes. The common signs and symptoms of major depressive episodes are outlined in the *Diagnostic and Statistical Manual, Fourth Edition, Text Revision* [1]. Although the neurobiology of depression is not well understood, several class of medications are used with depression, such as tricyclic antidepressants, tetracyclic antidepressants, monoamine oxidase inhibitors (MAOI), selective serotonin reuptake inhibitors (SSRI) and serotonin noradrenaline reuptake inhibitors (SNRI). Although these antidepressants are clearly beneficial for the treatment of major depression, they present many problems such as undesirable side-effects, relapse, refractory patients, and delayed onset of action. Consequently, there remains a pressing need for new antidepressant drugs.

In the present study, we investigated whether GLP-2 has antidepressant-like effects mostly using the forced-swim test (FST). The FST is one of the most widely used animal models of depression, in which an immobile posture except for those movements necessary to keep the head above the water line is induced when a rodent

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doi:10.1016/j.bbr.2009.06.020

has been placed in a tank of deep water for an extended period of time [41,42]. Immobility theoretically reflects lowered mood and helplessness induced by an inescapable stressor, and is usually facilitated by prior exposure to a swimming pretest or other stressors [6]. Administered chronically or after brief subchronic administration, antidepressant drugs decreased the duration of immobility in the FST [41,42].

Several experimental and clinical studies indicate that monoamine systems (serotonin: 5-HT, noradrenaline: NA, dopamine: DA) play a role in the pathophysiology of depression [22,33,38]. Drugs affecting the monoaminergic neurotransmission, such as those that inhibit monoamine reuptake at nerve terminals, or its metabolism (MAOI), are effective in depression. Also, early studies have shown that the depletion of monoamines with reserpine resulted in depressogenic effects in individuals already vulnerable to affective illness [23]. Although there is no direct evidence linking GLP-2 and the monoamine systems, GLP-2 receptor expressing sites are targets or nucleus origins of the monoamine systems. We thus evaluated the involvement of the monoamine systems in the effects of GLP-2 as well.

2. Materials and methods

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 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 8:00 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. Forced-swim test and Drug treatment

The test was performed by placing a mouse in an acrylic cylinder (50 cm tall, 18 cm in diameter) containing a 7 cm water column (25 °C). Water was replaced between every trial. Two swimming sessions were conducted: an initial 15-min pretest, followed by a 5-min test 24 h later. Test sessions were video-taped to measure the time of immobility, and immobility was defined as floating passively in the water and only making slight movements to keep the head above the water line.

The following drugs were used: GLP-2 (Peptide Inc., Osaka, Japan); prazosin, parachlorophenylalanine (PCPA) (Sigma, St. Louis, MO, USA), metergoline, NAN-190, atenolol, ICI118551, raclopride, SCH23394 (Tocris Cookson Ltd., Bristol, U.K.), yohimbine hydrochloride (Wako, Osaka, Japan).

GLP-2 was dissolved in 0.01 M phosphate-buffered saline (PBS). GLP-2 or PBS (the vehicle control) was administered into the lateral ventricular (i.c.v.) region of the mouse brain, since there was no report about the blood–brain barrier permeability of GLP-2. GLP-2 or PBS was administered 30 min before the FST sessions once a day for 2 days. The i.c.v. administration (a volume of 5 μl/mouse) was performed under brief ether anesthesia according to the method of Haley and McCormick [25]. PCPA was pretreated once a day for 4 consecutive days. Twenty-four hours after the last PCPA treatment, mice were administered with GLP-2 or PBS, and then FST was performed as described above. Most of pharmacological blockers were administered subcutaneously (s.c.) or intraperitoneally (i.p.) 30 min before GLP-2 administration. Only atenolol, which do not penetrate the blood–brain barrier, was administered into the lateral ventricle on the contralateral side of GLP-2 administration [14]. The administration routes and dose of each drug were described in the results section.

2.3. Locomotor activity

Locomotor activity was evaluated with the wheel running test and measured as the number of rotations (circumference 39 cm, Natsume, Tokyo, Japan). A counter was attached to each wheel, and data were recorded every 10 min for 60 min.

2.4. Tail suspension test (TST)

As in the FST, GLP-2 or PBS was administered to new mice two times 24 h apart. Thirteen minutes after the second administration of GLP-2 or PBS, mice were individually suspended by the tail to a horizontal ring-stand bar (distance from floor, 35 cm) using adhesive tape affixed 2 cm from the tip of the tail. Mice demonstrated several escape-oriented behaviors interspersed with bouts of immobility as the session progressed. A 5-min test session was videotaped, and the immobility time was measured as the time when mice were judged to cease escape-motivated behaviors.

2.4.1. Step-down type passive avoidance task

A step-down type passive avoidance task was used to evaluate the effects of GLP-2 on aversively motivated long-term memory performance. The apparatus consisted of a white acrylic rectangular cage (30 cm × 30 cm × 40 cm high) with a grid floor with a wooden platform (4 cm × 4 cm × 4 cm) in the center. Illumination was provided by a 15-W lamp above the apparatus. An electric current (1 Hz, 500 ms, 80 V DC) was delivered to the grid floor by an isolated stimulator (SEN-3201, Nihon Koden, Japan). Each mouse was placed on the wooden platform. Once the mouse stepped down from the platform onto the grid floor, an electric shock was delivered for 15 s. The retention test was carried out 24 h after the training session in a manner similar to the training except that no electric shock was delivered to the grid floor. Each mouse was placed on the platform and the time taken to step onto the grid floor (step-down latency) was recorded. A maximum latency of 300 s was set.

2.5. Data analysis

The immobility time is expressed as the mean ± S.E.M. In the FST, the significance of differences was evaluated using a parametric one-way analysis of variance (ANOVA) followed by Dunnett's or Bonferroni's multiple comparison test. If the variances of each group were unequal, a non-parametric Kruskal–Wallis test followed by Dunn's post hoc test was used. Mann–Whitney test and two-way ANOVA were used in the TST and the locomotor activity, respectively. All statistical analyses were performed using Graphpad Prism version 4 (Graphpad software Inc., San Diego, CA, USA). The criterion for significance was $P < 0.05$ in all statistical evaluation.

3. Results

3.1. Effects of GLP-2 on immobility time, locomotor activity and memory function in mice

Since a single administration of GLP-2 tended to have no effect on FST immobility time in our preliminary experiments (data not shown), GLP-2 was administrated once a day for 2 days in the present study. Administration of GLP-2 (1.5–6 μg/mouse, i.c.v.) once a day for 2 days significantly reduced FST immobility time in a dose-dependent manner (3 μg/mouse, 6 μg/mouse, Fig. 1A). However, GLP-2 did not affect locomotor activity in the wheel running test (Fig. 1B). To evaluate whether GLP-2 had antidepressant-like effects in another task, we also examined using the TST. Administration of GLP-2 (6 μg/mouse, i.c.v.) for 2 days significantly reduced the immobility time of the TST (Fig. 1C).

To exclude the possibility that GLP-2 inhibited memory function and erased the memory of aversive stimuli during the training session of the FST or the TST in mice, we evaluated the effects of GLP-2 on the memory function using a step-down type passive avoidance task. GLP-2 had no effects on the step-down latency (Fig. 1D).

3.2. Effects of pretreatment with antagonists of 5-HT receptors or an inhibitor of 5-HT synthase on the antidepressant-like effects of GLP-2 in mice FST

Fig. 2A and B shows the effects of pretreatment with antagonists of 5-HT receptors. Pretreatment with metergoline (6 mg/kg, s.c., Fig. 2A), an antagonist of non-selective 5-HT receptors, or NAN-190 (1 mg/kg, i.p., Fig. 2B), an antagonist of $5HT_{1A}$ receptors, significantly blocked the action of GLP-2 (6 μg/mouse, i.c.v.). Pretreatment with PCPA, an inhibitor of 5-HT synthase, also significantly inhibited the action of GLP-2 (Fig. 2C). Administration of PCPA alone had no effect on the immobility time.

3.3. Effects of pretreatment with antagonists of adrenoceptors on the antidepressant-like effects of GLP-2 in mice FST

Fig. 3 shows the effects of pretreatment with antagonists of adrenoceptors. Pretreatment with yohimbine (2 mg/kg, i.p.; Fig. 3A), an antagonist of α_2 receptors, or atenolol (300 nM/mouse, i.c.v.; Fig. 3B), an antagonist of β_1 receptors, significantly blocked the action of GLP-2 (6 μg/mouse, i.c.v.). However, neither pretreatment with prazosin (2 mg/kg, i.p.; Fig. 3C), an antagonist of α_1