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Antidepressant-like effect of food-derived pyroglutamyl peptides in mice

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ABSTRACT

The N-terminal glutamine residue, exposed by enzymatic cleavage of precursor proteins, is known to be modified to a pyroglutamyl residue with a cyclic structure in not only endogenous but also food-derived peptides. We investigated the effects of wheat-derived pyroglutamyl peptides on emotional behaviors. Pyroglutamyl leucine (pyroGlu-Leu, pEL) and pyroglutamyl glutaminyl leucine (pyroGlu-Gln-Leu, pEQL) exhibited antidepressant-like activity in the tail suspension and forced swim tests in mice. pEQL exhibited more potent antidepressant-like activity than pEL after i.p. and i.c.v. administration. pEQL exhibited antidepressant-like activity at a lower dose than Gln-Gln-Leu, suggesting that pyroglutamyl peptide had more potent activity. To examine whether pyroglutamyl peptides increased hippocampus neurogenesis, associated with the effects of antidepressants, we measured 5-bromo-2′-deoxyuridine (BrdU) incorporation. pEL and pEQL increased BrdU-positive cells in the dentate gyrus of the hippocampus. Intriguingly, pEL did not increase hippocampal mRNA and protein expression of brain-derived neurotrophic factor (BDNF), which is a factor associated with both neuropoietic and antidepressive effects. Thus, pyroglutamyl peptides may enhance hippocampal neurogenesis via a pathway independent of BDNF. We also confirmed that pEL and pEQL were produced in the subtilisin digest of major wheat proteins, glutenin and gliadin, after heat treatment. pEL and pEQL are the first peptides derived from wheat proteins to be shown to exhibit an antidepressant-like activity.

1. Introduction

A number of endogenous pyroglutamyl neuropeptides and peptide hormones, including neurotensin and thyrotropin-releasing hormone (TRH), have been discovered. The N-terminal glutamine residue, exposed by enzymatic cleavage of precursor proteins, is known to be enzymatically or automatically modified to a pyroglutamyl residue with a cyclic structure. These endogenous peptides, produced in the central nervous system (CNS), activate the target proteins to exhibit various physiological actions associated with emotional behavior, learning, pain response, food intake, metabolism, and so on. It was also reported that various bioactive peptides are released from food proteins after enzymatic digestion (Brantl et al., 1979; Yoshikawa et al., 1984). We previously reported that these exogenous peptides sometimes act on the nervous system to modulate emotional behavior, mimicking endogenous peptides (Hirata et al., 2007; Hou et al., 2011; Mizushige et al., 2013a; Oda et al., 2012; Ohinata et al., 2007b); however, little is known about the functions of pyroglutamyl peptides. We then focused on gluten, a major wheat protein, mainly consisting of glutenin and gliadin, which are rich in glutamine residues. In this study, we investigated the production of gluten-derived pyroglutamylated peptides and their effect on the emotional behavior in mice.

We found that tyrosyl leucine (Tyr-Leu, YL), a dipeptide consisting of an N-terminal cyclic amino acid and C-terminal Leu, exhibits anxiolytic-like activity in mice (Kanegawa et al., 2010; Mizushige et al., 2013b). It has recently been revealed that YL also exhibits antidepressant-like activity (Mizushige, unpublished observation). We then investigated whether wheat-derived pyroglutamyl peptides, including pyroGlu-Leu (pEL, Sato et al., 2013) and pyroGlu-Gln-Leu (pEQL), with structural similarity to YL, exhibit antidepressant-like effects in mice.

It is known that stress decreases hippocampal adult-neurogenesis and this neurogenesis can be increased by the administration of antidepressants in human and animals (Eisch and Petrik, 2012; Hanson et al., 2011; Malberg et al., 2000; Sahay and Hen, 2007; Santarelli et al., 2003). This so-called "neurogenesis hypothesis of depression" implies that neurogenesis and neuronal proliferation play a role in emotional regulation. It was reported that endogenous pyroglutamyl peptides stimulate neurogenesis (Kalivas and Horita,

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1980); however, the effects of food protein-derived peptides on neurogenesis and neuronal proliferation were still unknown. We thus investigated the effect of pyroglutamyl peptides on the hippocampal neuronal proliferation.

2. Materials and methods

2.1. Animals

Four- to five-week-old male ddY mice at 20–24 g or 24–30 g body weight for intracerebroventricular (i.c.v.) or intraperitoneal (i.p.) administration, respectively, were obtained from SLC (Shizuoka, Japan). All animals were housed in a temperature-controlled room (23 ± 1 °C) on a 12-h light–dark cycle with lights on at 07:00. All animals had free access to food pellets and water. Animals did not undergo more than one behavioral assay. The following biochemical experiments were performed using mice, which did not experience the behavioral test. All experiments were approved by Kyoto University Ethics Committee for Animal Research Use. All animals were euthanized by cervical spine dislocation after the experiment.

2.2. Reagents

PyroGlu-Leu (pEL), Gln-Leu (QL), pyroGlu-Gln-Leu (pEQL) and Gln-Gln-Leu (QQL) were synthesized by the F-moc method. Amino acids pyroGlu and Leu were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 5-Bromo-2′-deoxyuridine (BrdU) was from Sigma (St. Louis, MO, USA).

2.3. Tail suspension test (TST)

TST is a classical behavioral test for evaluating antidepressant-like activities. We performed the TST according to the method of Steru et al. with slight modification (Steru et al., 1985). Briefly, mice were suspended 30 cm above the floor using an adhesive tape placed approximately 2 cm from the extremity. Immobility time was recorded during a 6-min period. It was previously reported that the tests were performed during the light phase of the light/dark cycle (Ferrés-Coy et al., 2013; Nikulina et al., 1991; Zeidan et al., 2007). The TST was started at 11:00 am. Peptides dissolved in saline were i.p. administered 30 min before the test. We confirmed that the administration of imipramine, a typical antidepressant, decreased immobility time in the TST (saline-control and imipramine 30 mg/kg, 117 ± 9 and 12 ± 5 sec, respectively, $p < 0.05$; mean ± SEM, $n = 4$).

I.c.v. administration was performed as described previously (Kanegawa et al., 2010; Ohinata et al., 2007a, 2007b; Suzuki et al., 2011). Briefly, a 28-gauge stainless steel needle attached to a 0.05 mL Hamilton syringe was inserted perpendicularly through the skull into the brain. The site of injection was 2 mm from either side of the midline on a line drawn through the anterior base of the ears (lateral ventricle). pEL or pEQL was dissolved in artificial cerebrospinal fluid (ACSF; 138.9 mM NaCl, 3.4 mM KCl, 1.3 mM $CaCl_2$, 4.0 mM $NaHCO_3$, 0.6 mM NaH_2PO_4, 5.6 mM glucose, pH 7.4), and 4 μL was i.c.v. administered 20 min before the test, as previously described (Kanegawa et al., 2010; Ohinata et al., 2007a; Suzuki et al., 2011), since the effects of drugs after i.c.v. administration were generally assumed to be observed than those of i.p. I.c.v. administration was performed in TST but not in another test described below.

2.4. Forced swim test (FST)

FST is another behavioral test for evaluating antidepressant-like activity. It was performed according to the method of Porsolt et al. (1977). Briefly, mice were placed individually into glass cylinders (height 20 cm, diameter 10 cm) containing 8 cm of water at 25 ± 1 °C. They were left in the cylinder for 6 min and the total duration of immobility was measured using the SUPERMEX system (Muromachi Kikai, Co., Ltd., Tokyo, Japan). The water was changed after each trial. Peptides dissolved in saline were i.p. administered 30 min before the test. The forced swim test was started at 11:00 am during the light phase of the light/dark cycle. We also confirmed that administration of a typical antidepressant, imipramine, decreased the immobility time in the FST (data not shown).

2.5. BrdU incorporation in the hippocampus

pEL or pEQL (30 mg/kg) was i.p. administered three times (0, 3, 6 h), and BrdU (100 mg/kg) was i.p. administered 30 min after the first administration of pEL or pEQL. The doses were determined by preliminary experiments. Twenty-four hours after BrdU administration, mice were killed and transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde under pentobarbital anesthesia.

After perfusion, brains were post-fixed overnight in 4% paraformaldehyde at room temperature and dehydrated in 20% sucrose at 4 °C for 2 days. Serial sections of the brains were cut (40 μm sections) on a freezing microtome.

After washing the frozen sections with PBT (PBS with Triton X-100), they were incubated in 2 N HCl for 45 min and washed with PBT. After blocking in 3% normal donkey serum/1 M glycine PBT for 1 h, sections were incubated overnight with anti-rat BrdU (1:1000; Abcam, Cambridge, UK) at room temperature. Sections were incubated with the secondary antibody (1:500, donkey anti-rat Alexa 594; Life Technologies, Carlsbad, CA, USA) for 2 h. BrdU-labeled cells were counted at the hippocampus on an Olympus microscope.

2.6. Quantitative real-time RT-PCR for BDNF mRNA

pEL (30 mg/kg) was i.p. administered, and each mouse was decapitated 30 min after administration under deep anesthesia. The hippocampus was excised and kept in RNA later RNA Stabilization Reagent (QIAGEN Sciences Inc., Germantown, MD, USA) until RNA extraction. Hippocampal brain-derived neurotrophic factor (BDNF) mRNA expressions were measured by real-time RT-PCR. Total RNA was extracted from the hippocampus using the RNeasy Lipid Tissue Kit (QIAGEN Sciences Inc.) and transcribed to cDNA with random primers using Takara PrimeScript® RT Master Mix (Takara, Osaka, Japan). For quantitative PCR, we amplified the cDNA using the Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) with Platinum SYBR Green qPCR SuperMix-UDG and ROX solution (Invitrogen) and each primer set specific for mouse BDNF, according to the manufacturer's instructions. The following primers were used for real-time RT-PCR. Forward *bdnf*: 5′-GCG GCA GAT AAA AAG ACT GC-3′, Reverse *bdnf*: 5′-TCA GTT GGC CTT TGG ATA CC-3′, Forward *β-actin*: CTG CGC AAG TTA GGT TTT GTC A, Reverse *β-actin*: TGC TTC TAG GCG GAC TCT TAC TG. The reactions were cycled 40 times with denaturation at 95 °C for 15 s, and with annealing and elongation at 60 °C for 30 s. The relative expression level of each mRNA was normalized using the mRNA level of β-actin.

2.7. Quantitative determination of BDNF protein

Each hippocampus was excised 30 min after administration of pEL (30 mg/kg) under deep anesthesia and homogenized with ice-cold NP40 cell lysis buffer (Invitrogen). Blood was collected in a tube rinsed with heparin under deep anesthesia and centrifuged (1000 $g \times 10$ min 4 °C). The BDNF levels in the protein extract were measured using the Promega BDNF Emax® ImmunoAssay System (Promega, Madison, WI, USA) according to the manufacturer's instructions.