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Short communication

Enterostatin (APGPR) enhances memory consolidation in mice

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ABSTRACT

Enterostatin (APGPR) is a pentapeptide released from its precursor protein, procolipase. We found for the first time that enterostatin has memory-enhancing activity. Enterostatin enhanced memory consolidation after central or oral administration at a dose of 10 nmol/mouse or 300 mg/kg, respectively, in a step-through type passive avoidance test in mice. The memory-enhancing activity of enterostatin was inhibited by pretreatment with lorglumide, an antagonist for cholecystokinin 1 (CCK_1) receptor. However, enterostatin had no affinity for CCK receptors. These results suggest that enterostatin improves memory retention through CCK release.

1. Introduction

Enterostatin, a pentapeptide released from the N-terminus of pancreatic procolipase by proteolytic activation in the small intestine, decreases fat intake after central and peripheral administration [1,3,4]. The procolipase gene is expressed in the gastric and duodenal mucosa, and the central nervous system (CNS) in addition to the exocrine pancreas [10,15]. The immunoreactivity of enterostatin is also located at similar sites, suggesting that procolipase is processed in the gastrointestinal tract and the CNS [11,15].

Enterostatin has a number of physiological functions such as suppression of fat intake, inhibition of insulin secretion and activation of sympathetic nerve activity [1,3,4]. We previously reported that enterostatin analogue (VPDPR) improves scopolamine-induced amnesia [13]. In this study, we found that human enterostatin (APGPR) had memory-enhancing activity after central or oral administration and investigated the mechanism of memory consolidation by enterostatin.

CCK is a well-known satiety signal molecule and is found both in the CNS and the gastrointestinal tract [8,12]. In the brain, CCK acts as a neurotransmitter [8,12]. Two receptor subtypes for CCK (CCK_1 and CCK_2 receptors) are known. It has been reported that enterostatin inhibits fat intake through cholecystokin (CCK) release followed by CCK_1 receptor activation [7]. CCK_1 receptor is associated with food intake suppression and memory consolidation [8,9,12]. Thus, we also investigated whether the memory enhancement of enterostatin was mediated by the CCK_1 receptor.

2. Materials and methods

2.1. Reagents

APGPR was synthesized by the Fmoc strategy. Lorglumide, an antagonist for CCK_1 receptor, was obtained from Sigma–Aldrich Co. (St. Louis, MO).

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2.2. Animals

Four-week-old male ddY mice (SLC, Shizuoka, Japan) were used. Mice were housed under regulated conditions (22 °C on a 12 h light-dark cycle, lights on 07:00–19:00), and fed food pellets and water *ad libitum*. All experiments were approved by the University Animal Committee.

2.3. Step-through-type passive avoidance test

The passive avoidance test was performed as described previously [14]. Briefly, the step-through apparatus consisted of light and dark compartments with a grid floor made of stainless steel rods. These rooms were connected by a hole. In the training trial, each mouse was placed into the illuminated room. Mice enter the dark compartment, because they are nocturnal. Then, an electric shock (0.12–0.14 mA) was given until the mouse returned to the light room. Just after the training trial, enterostatin was intracerebroventricularly (i.c.v.) or orally administrated [14]. The test trial by placing the mouse again into the light room was performed 24 h after training. The latency time passed in the light compartment (step-through latency) was measured. The cutoff was set at 600 s. I.c.v. administration into the lateral ventricle was performed as described previously [14]. Enterostatin dissolved in 4 μl 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) was injected into the lateral ventricle.

2.4. Statistical analysis

Data from the passive avoidance test using step-through apparatus were expressed as the median and interquartile ranges. Mann–Whitney's *U*-test was used for comparisons between groups and *P* values less than 0.05 were considered significant.

3. Results

3.1. Enterostatin enhances memory consolidation

Orally administered enterostatin at a dose of 300 mg/kg increased step-through latency in a dose-dependent manner as shown in Fig. 1a. Central administration of enterostatin at a dose of 10 nmol/mouse also increased step-thorough latency (Fig. 1b). Thus, enterostatin had memory-enhancing activity after oral and central administration in normal mice.

3.2. Memory-enhancing activity of enterostatin was mediated by CCK_1 receptor

Pretreatment with lorglumide, an antagonist for CCK_1 receptor (0.1 mg/kg, i.p.) 15 min before the administration of enterostatin (10 nmol/mouse, i.c.v.) completely abolished the enhancement of memory consolidation by enterostatin (Fig. 2). However, enterostatin did not show affinity for CCK_1 receptor (data not shown). Taken together, enterostatin enhances memory consolidation through CCK release followed by the activation of CCK_1 receptor.

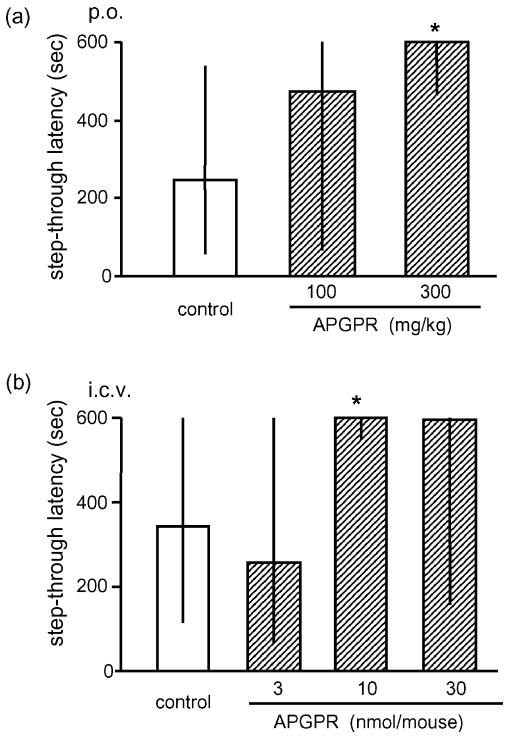

Fig. 1 – Effect of oral or central administration of APGPR on memory consolidation. APGPR at a dose of 100–300 mg/kg p.o. (a) or 3–30 nmol/mouse i.c.v. (b) was given immediately after training. Each value represents the median and interquartile ranges ((a) *n* = 10, (b) *n* = 8–19). *P < 0.05 compared with control group, Mann–Whitney's *U*-test.

4. Discussion

We found that enterostatin improves step-through latency in passive avoidance test after oral and central administration in normal mice. Centrally administered enterostatin just after

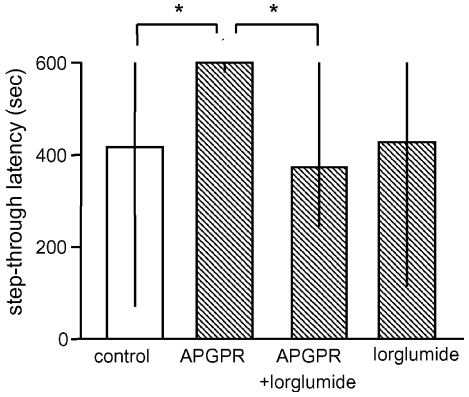

Fig. 2 – Effect of a CCK_1 receptor antagonist lorglumide on the enhancement of memory consolidation of APGPR. Lorglumide (0.1 mg/kg, i.p.) was given 15 min before training, and APGPR (10 nmol/mouse, i.c.v.) was given immediately after training. Each value represents the median and interquartile ranges (*n* = 7). *P < 0.05 compared with each group, Mann–Whitney's *U*-test.

training improved step-through latency, however, 2 h after training it was inactive (data not shown), suggesting that enterostatin enhances memory consolidation. Two signaling pathways of orally administered enterostatin to the CNS are hypothesized. One is a neuronal pathway via the afferent vagus. Inhibition of high-fat diet consumption with intraperitoneal enterostatin was completely blocked by vagotomy, suggesting that the signal of peripheral enterostatin is neuronally transmitted to the CNS [3]. Another is a direct pathway. Although not a large amount, enterostatin is able to cross the blood brain barrier (BBB) [6]. At present, it is not clear whether the memory-enhancing effect of orally administered enterostatin is mediated by the vagal pathway or enterostatin directly passing across the BBB.

Enterostatin induced memory-enhancing activity was completely blocked by a CCK_1 receptor antagonist, lorglumide. However, enterostatin did not have affinity for the CCK_1 receptor. Thus, the memory-enhancing effect is mediated through CCK release followed by the activation of CCK_1 receptor.

In the brain, CCK is one of the most abundant neurotransmitter peptides [2,9]. Relatively high concentrations of CCK exist in the hippocampus and frontal cortex, which is associated with memory and learning [9]. It has been reported that the suppression of fat intake by enterostatin was blocked by lorglumide, and enterostatin did not decrease fat intake in Otsuka Long Evans Tokushima Fatty (OLETF) rats lacking CCK_1 receptor [7]. Memory and learning are impaired in OLETF rats without CCK_1 receptor [9]. These reports are consistent with our results that enterostatin enhances memory consolidation through the CCK_1 receptor.

It has been reported that anorexigenic peptides and proteins such as leptin, CCK and corticotrophin releasing factor (CRF) increase learning performance [5,9,16]. In this study, we found that enterostatin having anorexigenic activity enhanced memory consolidation. Enterostatin is new example of anorexigenic peptides improving memory retention.

Although a high dose was necessary, enterostatin may be a candidate for orally effective pharmaceutical drugs to enhance memory consolidation. In addition, we are incorporating the enterostatin sequences into soy bean protein. If the functional protein is abundantly produced in soy beans, it can be inexpensively utilized as a beneficial food.

In conclusion, we found that orally and centrally administered enterostatin had memory-enhancing activity, which was mediated by CCK_1 receptor.

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