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Short Communication

Growth Hormone Releaser Attenuates β-Amyloid (1 – 42)-Induced Memory Impairment in Mice

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Abstract. Accumulating evidence indicates that growth hormone (GH) might be effective at preventing the development of Alzheimer's disease. However, exogenous GH treatment has exhibited side effects for clinical application; thus supplementation with amino acids to promote the release of GH could be a possible alternative treatment. In this study, mice that were fed with a diet of GH-releasing supplements had significantly attenuated memory impairments and hippocampal changes in the acetylcholinesterase activity and acetylcholine level induced by amyloid beta protein (Aβ) (1 – 42). Our results suggest that the use of GH-releasing supplement exerts beneficial effects on the memory impairment induced by Aβ (1 – 42).

Keywords: growth hormone releaser, amyloid beta protein, cognitive enhancer

The activities of growth hormone (GH) and insulin-like growth factor-I (IGF-I) are stimulated by GH and declined with aging (1), and this is known to contribute to age-related cognitive performance decay (2).

Rivastigmine, a drug for Alzheimer's disease (AD), was found to enhance GH release (3). This finding suggests that GH administration might be effective at preventing the development or progression of AD. However, exogenous GH treatment in healthy elderly subjects or AD patients is not an attractive tool because of its potentially harmful side effects, requirement of injection, and high cost (4). Thus, the methods for increasing the endogenous GH secretion and subsequent IGF-I synthesis by oral intake might be a better alternative. As the oral administration of amino acids (i.e., arginine, glutamine, glycine, and lysine) has been found to increase the release of endogenous GH (5), supplementation with these amino acids might be a beneficial pharmacological intervention.

In this study, we examined whether GH-releasing supplements could be effective in protecting against the memory deficits and changes in acetylcholine (ACh) levels and acetylcholinesterase (AChE) activity induced by intracerebroventricular (i.c.v.) injection of amyloid beta protein (Aβ) (1 – 42) in mice.

All of the animals used in this study were treated in strict accordance with the NIH Guide for the Humane Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1985). This study was performed in accordance with ILAR guideline for the care and use of laboratory animals. Male C57BL/6 mice weighing about 30 ± 2 g were maintained on a 12:12 h light:dark cycle and fed ad libitum. The Aβ (1 – 42) (U.S. Peptide, Fullerton, CA, USA) and Aβ (40 – 1) (Bachem, Torrance, CA, USA) were dissolved in 35% acetonitrile containing 0.1% trifluoroacetic acid. The Aβ (1 – 42) or Aβ (40 – 1) administration [400 pmol, i.c.v. injection; Aβ (40 – 1) was used for the control of Aβ (1 – 42), because Aβ (42 – 1) is not available (6, 7)], was per-

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formed according to the procedure established by previous reports (6). Briefly, each mouse was injected at the bregma with a 50-μl Hamilton microsyringe fitted with a 26-guage needle that was inserted to a depth of 2.4 mm. The injection volume was 5 μl.

The experimental diet was composed of 5% GH releaser (a mixture of L-arginine, L-glutamine, L-lysine, and glycine at a ratio of 37:30:18.5:14.5), 45% sucrose, 20% casein, 15% corn starch, 7% corn oil, 3% cellulose, 3% mineral mixture, 1.5% vitamin mixture, 0.3% DL-methione, and 0.2% choline bitartrate (8). The composition ratio of amino acids was comparable to that in our previous study (9). The experimental schedule is shown in Fig. 1. Mice received the GH releaser-containing diet for 20 day before Aβ (1 – 42) i.c.v. injection and continued on the diet throughout the experimental period. The behavioral study was begun on day 3 after Aβ (1 – 42) i.c.v. injection and conducted sequentially. Mice were sacrificed at 4 day after Aβ (1 – 42) i.c.v. injection to examine Ach levels and AChE activity (Fig. 1).

Since the positive effect of GH releaser was already observed by the Y-maze test in the preliminary study (9), the learning and memory capacities were evaluated with a water maze test and passive avoidance test in this study according to the procedure of our recent study (6). GH level was measured in serum samples by using a GH EIA kit (American Laboratory Products, Windham, NH, USA) (10). ACh levels were examined as described by Israel and Lesbats (11), and AChE activity in the hippocampal homogenates was assayed by the Ellman method (12).

The statistical significance of the behavioral and biochemical effects of GH releaser was determined by one-way analysis of variance (ANOVA) followed by the Bonferroni's test or ANOVA with Duncan's new multiple (DMR) test. Two-way ANOVA was also conducted for analyzing data of escape latency for reference memory of the water maze test.

The changes in the escape latency to the hidden platform produced by training trials in each group of mice are shown in Fig. 2A. Two-way ANOVA conducted on all of the treatment groups showed significant group ($F_{3,576} = 19.906$, $P<0.01$) and training ($F_{15,576} = 19.418$, $P<0.01$) effects, but no group by trial interactions ($F_{45,476} = 1.225$, $P>0.05$). The *Post-hoc* test indicated that performance in the Aβ (1 – 42)-treated group was significantly impaired compared with that in the Aβ (40 – 1)-treated group ($P<0.01$). The prolonged exposure to GH releaser significantly ameliorated the impairment of performance caused by Aβ (1 – 42) injection into the cerebral ventricle ($P<0.01$). However, the prolonged exposure to GH releaser did not show any changes in the Aβ (40 – 1)-treated group. The reduced number of annulus crossings in the Aβ (1 – 42)-treated mice as compared with Aβ (40 – 1)-treated mice ($F_{1,18} = 5.671$, $P<0.05$) was also significantly reversed ($F_{1,18} = 6.227$, $P<0.05$) by GH releaser (Fig. 2B). The mean escape latency measured for working memory was significantly longer in the Aβ (1 – 42)-treated mice than in the Aβ (40 – 1)-treated mice ($F_{1,238} = 22.175$, $P<0.01$). GH releaser also mitigated significantly Aβ (1 – 42)-induced impairment of performance in the working memory test ($F_{1,238} = 4.569$, $P<0.05$) (Fig. 2C).

The step-through latency in the retention trial was significantly decreased ($F_{1,28} = 13.315$, $P<0.01$) in the

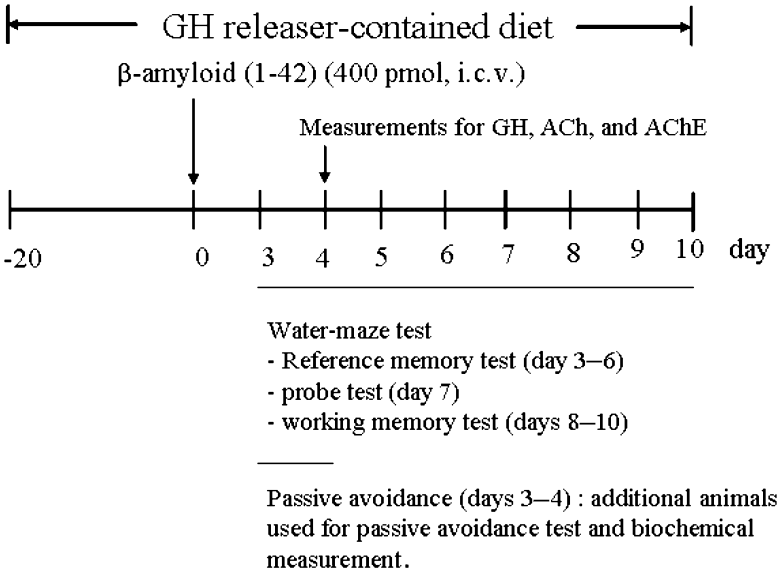

Fig. 1. Experimental schedule. GH, growth hormone; ACh, acetylcholine; AChE, acetylcholinesterase.

Aβ (1 – 42)-treated mice compared with those in the Aβ (40 – 1)-treated mice. Additionally, the step-through latency of the Aβ (1 – 42)-treated mice that were fed GH releaser was significantly longer ($F_{1,28} = 4.534$, $P<0.05$) than the latency of the Aβ (1 – 42)-treated mice that were not fed GH releaser (Fig. 2D).

The hippocampal ACh levels significantly decreased ($F_{1,28} = 7.203$, $P<0.05$) in the Aβ (1 – 42)-treated mice compared with the levels in the Aβ (40 – 1)-treated mice, but this reduction was attenuated significantly ($F_{1,28} = 4.472$, $P<0.05$) by GH releaser. Serum level of GH showed a decreased pattern in the Aβ (1 – 42)-treated mice. Exposure to GH releaser was significantly enhanced ($F_{1,10} = 5.116$, $P<0.05$) the GH level of Aβ (1 – 42)-treated mice. Since the effect of 5% GH releaser is almost equi-potent to that of 10% GH releaser in releasing GH in the preliminary study, we applied 5% GH releaser in this study. GH level in the Aβ (40 – 1)-treated mice was significantly increased ($F_{1,10} = 5.157$, $P<0.05$) by GH releaser. Similarly, ACh levels appeared to increase in this group as compared with Aβ (40 – 1) alone (Fig. 3A).

AChE activities consistently and significantly increased ($F_{1,28} = 5.281$, $P<0.05$) in the Aβ (1 – 42)-treated mice as compared with activities in the Aβ (40 – 1)-treated mice, but this increase was reduced significantly ($F_{1,28} = 4.313$, $P<0.05$) in the presence of GH releaser (Fig. 3B). Furthermore, our previous reports suggested that Aβ (1 – 42) decreases hippocampal choline acetyl transferase activity (7). Therefore, the hippocampal level of ACh may decrease after Aβ (1 – 42) insult.

We demonstrated that GH releaser attenuates memory deficits induced by a single i.c.v. Aβ injection in mice. To our knowledge, this is the first time that a GH releaser has been shown to attenuate memory impairment and cholinergic deficits in vivo. However, GH releaser failed to increase ability of learning and memory in control animals [serum level of IGF-1 was not significantly increased in the group of Aβ (40 – 1) plus GH releaser (170 ± 32 μg/l; n = 6) as compared with that of Aβ (40 – 1) alone (158 ± 25 μg/l; n = 6)], since GH levels are under the homeostatic regulation in normal physical condition. Therefore, it may have protective or therapeutic effects on dementia. Because GH releaser did not show any potential serious side effects, which are seen in the case of exogenous GH

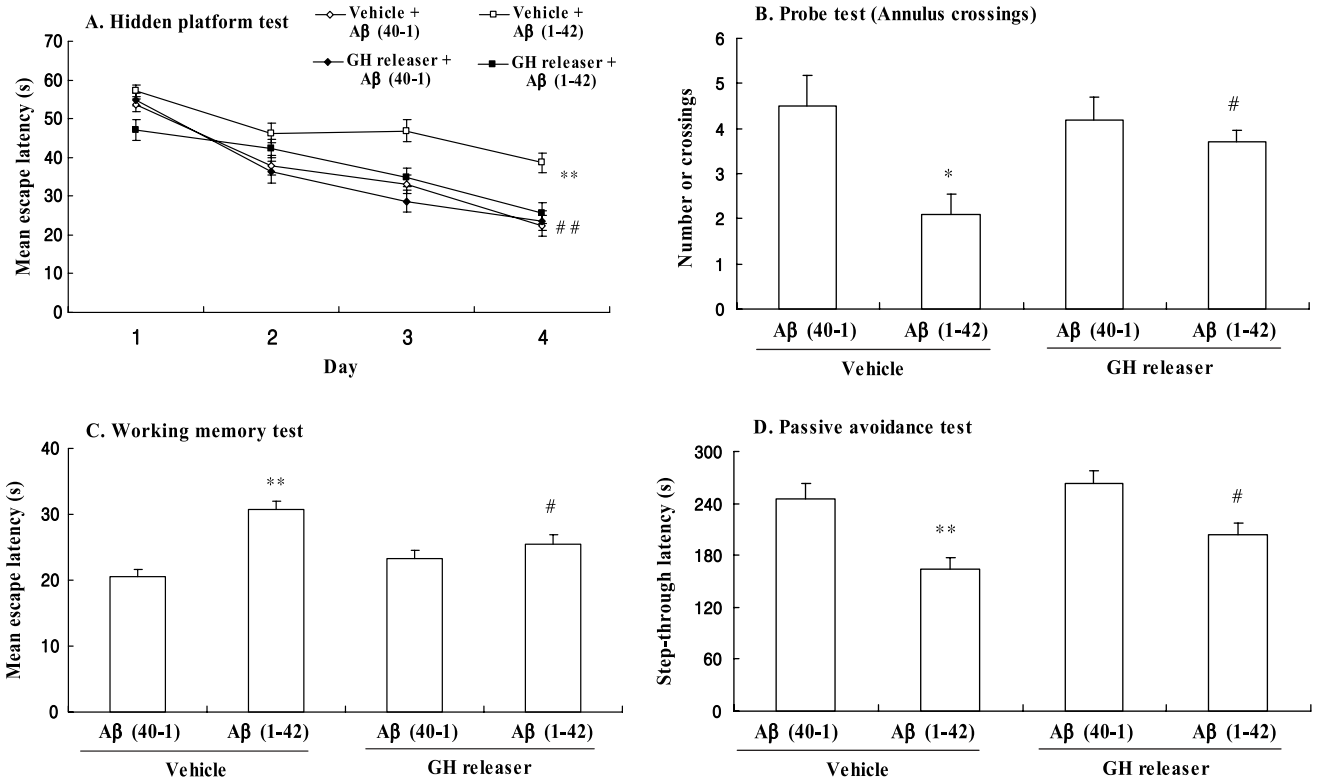

Fig. 2. The effects of GH releaser on the performance of Aβ (1 – 42)-treated mice in the training (A), probe test (B), working memory trial (C) of the water maze test, and retention (D) of the passive avoidance task. Each value is the mean ± S.E.M. of 10 (water maze test) or 15 animals (passive avoidance test). **P*<0.05 or ***P*<0.01 *vs* Vehicle + Aβ (40 – 1), $^{\#}P<0.05$ or $^{\#\#}P<0.01$ *vs* Vehicle + Aβ (1 – 42). The training (A) was determined by One-way ANOVA followed by Bonferroni's test, and statistical analyses for B – D were performed by One-way ANOVA with the DMR test.

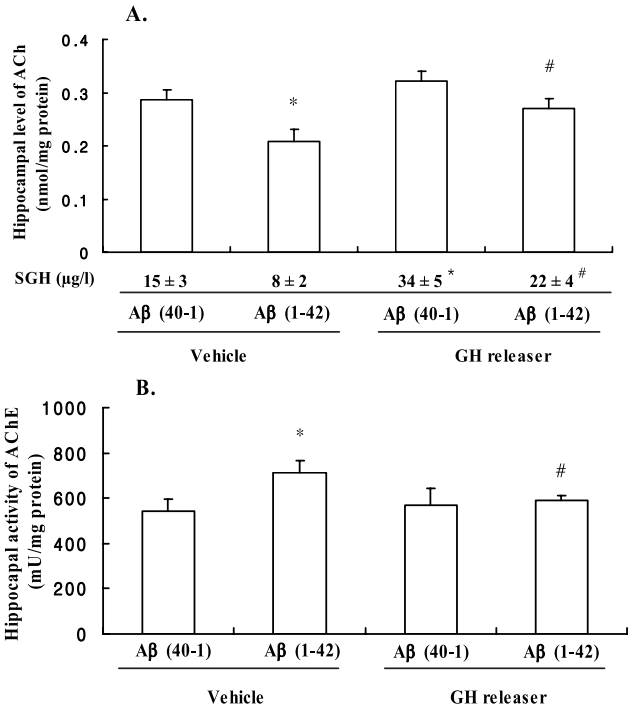

Fig. 3. Effects of GH releaser on the changes in the GH level, ACh level (A), and AChE activity (B) induced by Aβ (1 – 42) in mice. SGH = serum growth hormone level. Each value is the mean ± S.E.M. of 6 (for SGH) or 15 (for hippocampal-ACh level and -AChE activity) animals. *P<0.05 *vs* Vehicle + Aβ (40 – 1), $^{\#}P$<0.05 *vs* Vehicle + Aβ (1 – 42) (One-way ANOVA with DMR test).

treatment (4), our diet formulation may provide a starting point for the development of an effective oral GH releaser for the clinical application. The immunocytochemical analysis showed Aβ-like immunoreactivity in the hippocampus after i.c.v. Aβ (1 – 42) injection (13), although it remains to be fully characterized on the hippocampal status of Aβ. It was suggested that GH-stimulated IGF-1 expression is regulated by the STAT5b pathway (14) and that a muscarinic cholinergic mechanism regulates GH secretion (15).

We propose that GH releaser-induced GH might affect IGF-1 signaling for attenuating memory impairment induced by Aβ, but more evidence should be gathered.

Acknowledgments

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References

1 Lamberts SW, van den Beld AW, van der Lely AJ. The endocrinology of aging. Science. 1997;278:419–424.
2 van Dam PS, Aleman A, de Vries WR, Deijen JB, van der Veen EA, de Haan EH, et al. Growth hormone, insulin-like growth factor I and cognitive function in adults. Growth Horm IGF Res. 2000;Suppl B:S69–S73.
3 Obermayr RP, Mayerhofer L, Knechtelsdorfer M, Tragl KH, Geyer G. The reduced release of GH by GHRH in 8 subjects aged 65-69 years is augmented considerably by rivastigmine, a drug for Alzheimer's disease. Gerontology. 2003;49:191–195.
4 Khorram O. Use of growth hormone and growth hormone secretagogues in aging: help or harm. Clin Obstet Gynecol. 2001;44:893–901.
5 Chromiak JA, Antonio J. Use of amino acids as growth hormone-releasing agents by athletes. Nutrition. 2002;18:657–661.
6 Jhoo JH, Kim HC, Nabeshima T, Yamada K, Shin EJ, Jhoo WK, et al. beta-Amyloid (1-42)-induced learning and memory deficits in mice: involvement of oxidative burdens in the hippocampus and cerebral cortex. Behav Brain Res. 2004;155:185–196.
7 Yamada K, Tanaka T, Mamiya T, Shiotani T, Kameyama T, Nabeshima T. Improvement by nefiracetam of β-amyloid-(1-42)-induced learning and memory impairments in rats. Br J Pharmacol. 1999;126:235–244.
8 National Research Council. Neutrient requirement of laboratory animals-mouse. Washington DC: National Academic Press; 1978; p. 38–53.
9 Kwon MS, Shin EJ, Nabeshima T, Kim YS, Park SJ, Jin CH, et al. Growth hormone booster attenuates memory impairment and oxidative stress in the genetic model of aging gene Klotho mutant mice. Society for Neuroscience. 2004;34:Abstract No 450.10.
10 Sun Y, Wang P, Zheng H, Smith RG. Ghrelin stimulation of growth hormone re;ease and appetite is mediated through the growth hormone secretagogue receptor. Proc Natl Acad Sci USA. 2004;101:4679–4684.
11 Israel M, Lesbats B. Application to mammalian tissue of the chemiluminiscent method for detecting acetylcholine. J Neurochem. 1982;39:248–250.
12 Moya-Quiles MR, Villalba-Sanchez J, Munoz-Delgado E, Vidal CJ. Alkaline treatment of muscle microsomes releases amphiphilic and hydrophilic forms acetylcholinesterase. Biochim Biophys Acta. 1992;1121:88–96.
13 Nitta A, Fukuta T, Hasegawa T, Nabeshima T. Continous infusion of β-amyloid protein into cerebral ventricle induces learning impairment and neuronal and morphological degeneration. Jpn J Pharmacol. 1997;73:51–57.
14 Woelfle J, Chia DJ, Rotwein P. Mechanism of growth hormone (GH) action. J Biol Chem. 2003;278:51261–51266.
15 Brillion D, Nabil N, Jacobs LS. Cholinergic but not serotonergic mediation of exercise-induced growth hormone secretion. Endocr Res. 1986;12:137–146.