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Rescue of cognitive-aging by administration of a neurogenic and/or neurotrophic compound

Silvia Bolognin [a,1], Mario Buffelli [a,1], Jukka Puoliväli [b], Khalid Iqbal [a,*]

[a] Department of Neurochemistry, Inge Grundke-Iqbal Research Floor, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA
[b] Department of Behavioral Studies, Charles River Finland, Kuopio, Finland

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ABSTRACT

Aging is characterized by a progressive decline of cognitive performance, which has been partially attributed to structural and functional alterations of hippocampus. Importantly, aging is the major risk factor for the development of neurodegenerative diseases, especially Alzheimer's disease. An important therapeutic approach to counteract the age-associated memory dysfunctions is to maintain an appropriate microenvironment for successful neurogenesis and synaptic plasticity. In this study, we show that chronic oral administration of peptide 021 (P021), a small peptidergic neurotrophic compound derived from the ciliary neurotrophic factor, significantly reduced the age-dependent decline in learning and memory in 22 to 24-month-old Fisher rats. Treatment with P021 inhibited the deficit in neurogenesis in the aged rats and increased the expression of brain derived neurotrophic factor. Furthermore, P021 restored synaptic deficits both in the cortex and the hippocampus. In vivo magnetic resonance spectroscopy revealed age-dependent alterations in hippocampal content of several metabolites. Remarkably, P021 was effective in significantly reducing myoinositol (INS) concentration, which was increased in aged compared with young rats. These findings suggest that stimulating endogenous neuroprotective mechanisms is a potential therapeutic approach to cognitive aging, Alzheimer's disease, and associated neurodegenerative disorders and P021 is a promising compound for this purpose.

1. Introduction

Aging, though physiological in nature, is considered as a critical condition characterized by a progressive deterioration of the overall homeostatic brain mechanisms. In humans, cerebral aging implies a variety of morphologic alterations that include enlargement of ventricles, progressive loss of brain weight (Bertoni-Freddari et al., 2008), and histopathologically significant reduction in the number of synapses (Burke and Barnes, 2006). Hippocampus is the key target of these age-associated changes that affect both its structural and functional integrity, resulting in learning and memory deficits (Driscoll and Sutherland, 2005; Rosenzweig and Barnes, 2003). Aging is also the most important risk factor for the development of neurodegenerative diseases such as Alzheimer's disease (AD). This has mainly been attributed to alterations of cell microenvironment (neurogenic niche), which compromise the brain milieu. One of the most direct effects of this insufficient microenvironment support is the dramatic decrease of proliferative activity in the aging brain (Drapeau and Nora Abrous, 2008; Miranda et al., 2012). Indeed, the neuronal survival is thought to depend on the surrounding neurotrophic microenvironment. Furthermore, the propensity of newly generated cells to adopt a neuronal phenotype (successful neurogenesis) markedly diminishes with aging (Driscoll et al., 2006; Heine et al., 2004).

It is now increasingly believed that adult-generated neurons contribute to the formation of hippocampal-dependent memory (Deng et al., 2009; Shors, 2008) and these cells can be integrated into patterns of memory networks. Notably, evidence suggests that neurons formed during development and adulthood in the dentate gyrus (DG) are most likely integrated into the hippocampal memory circuits at the same rates and equally contribute to hippocampal memory formation (Stone et al., 2011). Accordingly, adult neurogenesis suppression was demonstrated to affect different forms of hippocampal-dependent learning, such as

* Corresponding author at: Department of Neurochemistry, Inge Grundke-Iqbal Research Floor, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314, USA. Tel.: +1 718 494 5259; fax: +1 718 494 1080.
E-mail address: khalid.iqbal.ibr@gmail.com (K. Iqbal).
[1] Present address: Department of Neurological and Movement Sciences, Section of Physiology University of Verona, Strada le Grazie 8, 37134 Verona, Italy.

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Morris water maze and contextual fear conditioning in rodents (Deng et al., 2009; Shors, 2008). Thus, a decline of adult neurogenesis has clearly been correlated to a decline of hippocampal memory functions.

The mechanisms underpinning the decrease in successful neurogenesis in the DG are still not well understood (Lazarov and Marr, 2013). However, up-regulation of signals suppressing self-renewal of neural stem cells (Bonaguidi et al., 2008) or decreased neurotrophic factor levels (Bernal and Peterson, 2011; Hattiangady et al., 2005; Shetty et al., 2005) have been both hypothesized as potential causative factors. Consequently, the approaches aiming at recovering the biochemical milieu of the brain might be a promising therapeutic option to enhance healthy aging and eventually to counteract neurodegeneration, especially that seen in AD. Toward this direction, several studies showed the key role of neurotrophins in the promotion on neuronal survival and modulation of synaptic connectivity (Dawbarn and Allen, 2003; Rosenblad, 2004). Among these factors, ciliary neurotrophic factor (CNTF) has shown remarkable neuroprotective properties (Chojnacki et al., 2003; Song et al., 2002).

We have previously shown in cell culture that CNTF counteracted the effect of increased fibroblast growth factor-2, which impairs neuronal lineage determination and maturation, resulting in promotion of successful neurogenesis (Chen et al., 2001, 2007). However, attempts to use CNTF (as other neurotrophic factors) as an effective tool to delay deterioration of hippocampal-function have been so far inconclusive. This was mainly because of the lack of effective delivery systems as peripherally administered CNTF poorly reached the central nervous system (Chen et al., 2001) and to the appearance of serious side effects such as anorexia, hyperalgesia, muscle loss, and pain. The use of small CNTF-derived peptides, showing the same neuroprotective properties of trophic factor but without the limitations mentioned previously, represents an important therapeutic challenge.

We have previously demonstrated that peripheral administration of a blood–brain-barrier-permeable 11 mer peptide, peptide 6, corresponding to the active region of CNTF (amino acid residues 146–156) promoted DG neurogenesis and increased synaptic protein expression, which rescued behavioral impairment in rodent models of sporadic (Bolognin et al., 2012) and familial AD (Blanchard et al., 2010b) and Down syndrome (Blanchard et al., 2011). This positive effect of the peptide was mainly achieved by antagonizing the activity of leukemia inhibitory factor and by increasing the transcription of brain derived neurotrophic factor (BDNF) (Bolognin et al., 2012; Chohan et al., 2011). Moreover, peptide 6 improved cognitive performance in normal adult mice (Chohan et al., 2011). We further reduced the active region of peptide 6 to only 4 amino acids and we added a C-terminally adamantylated group to increase the lipophilicity and stability of the tetrapeptide, which we called peptide 021 (P021) or Compound 021 (DGGL^{A}G). In previous studies both P021 and its parent non-adamantylated peptide increased neurogenesis, synaptic marker expression, and improved cognitive performance in normal adult mice (Blanchard et al., 2010a; Li et al., 2010).

The present study shows that oral chronic treatment with Compound P021 for 88 days can rescue age-associated neurogenesis and neuronal plasticity deficits and cognitive impairment in aged female Fisher rats. These positive effects of P021 involved increase in the expression of BDNF and activation of its signaling pathway as well as increase in synaptic activity both in the cortex and hippocampus. Using in vivo magnetic resonance spectroscopy (MRS), we also detected age-dependent alterations in the hippocampal content of several metabolites. Remarkably, P021 was effective in significantly reducing myo-inositol (INS) level, which was increased in the aged rats.

2. Methods

2.1. Structure of P021 and study outline

Female aged (19–21 months) Fischer rats were given P021 (Fig. 1A) per os by gavage (10 mL/kg body weight) once a day for 88 days (Fig. 1B). The dose of P021 was 500 nanomoles 289.15 μg/kg body weight daily. As controls, a second group of aged, and a group of young adult (2–3 months) rats were identically treated but with vehicle (normal saline) only. Administration of vehicle and test compound was done at 7–9 AM daily in the pretesting phase. On the days of behavioral testing, the treatment was given minimum 1 hour before the first test trial. At the end of the treatment, the effect of P021 administration was tested by a spatial reference memory task, in vivo 1H-MRS and fluorodeoxyglucose positron emission tomography (FDG-PET). After sacrificing the animals, tissue was processed and assessed for neurogenesis, BDNF-pathway related proteins, synaptic, and dendritic markers. To detect neurogenesis, the cell proliferation specific marker 5-bromodeoxyuridine (BrdU, Sigma) was administered (50 mg/kg BrdU dissolved in saline, intraperitoneally, 5 mL/kg). BrdU injections were started on day 45 and continued once a day for 5 days. Female Fisher rats were

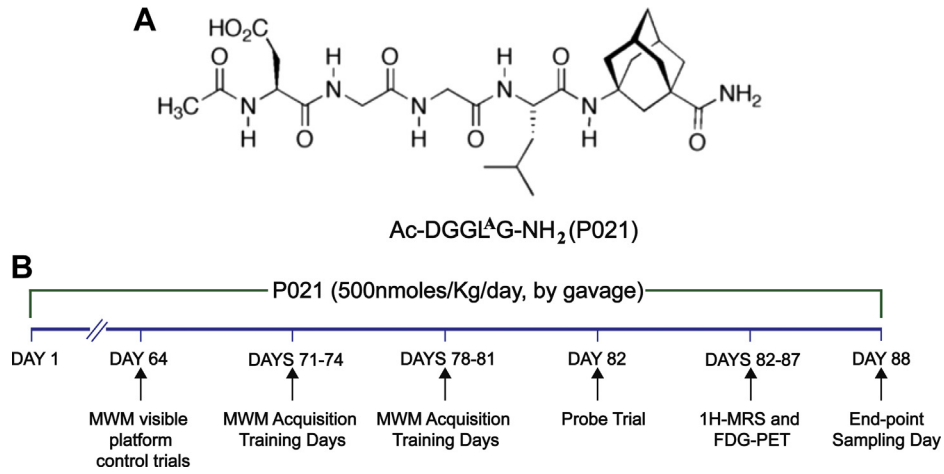

Fig. 1. Schematic representation of the outline of the study. Chemical structure of P021 (A) and design of the study (B).