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Hippocampal CREB1 but not CREB2 is decreased in aged rats with spatial memory impairments☆

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Abstract

Recent evidence has shown that abnormal signal transduction is related to non-pathological memory impairment among aged subjects. Members of the CREB family of transcription factors contain enhancers (i.e., CREB1) and repressors (i.e., CREB2) of transcription and interact with numerous signaling proteins to mediate the transition from short-term to long-term memory. In this study, quantitative Western blotting was used to determine the levels of CREB1 and CREB2 in homogenates from hippocampi of individual 6- and 24-month-old male Long-Evans rats trained first on a place-learning task in the Morris water maze, then on a transfer task. Based on spatial memory performance, aged rats were characterized into two groups; aged-unimpaired rats (AU) had scores within the range of the young (Y) and aged-impaired rats (AI) fell outside of that range. Overall, CREB1 protein was significantly lower in aged rats in comparison with young rats. *Aposteriori* analysis showed that this difference was due to a significant decrease in CREB1 levels among aged-impaired rats, whereas aged-unimpaired rats had CREB1 levels comparable to young rats. There was no significant change in levels of CREB2 protein between young and aged rats. These results show that the dysregulation of CREB1 protein may contribute to the spatial memory deficits observed among some aged subjects.

Keywords: Transcription factor; Morris water maze; Memory; CREB; Aging

1. Introduction

Aged rats show deficits in a variety of hippocampal-dependent spatial memory tasks, including the radial arm maze (de Toledo-Morrell, Geinisman, & Morrell, 1988), the Barnes circular maze (Barnes, 1988), and the Morris water maze (Gallagher & Pelleymounter, 1988). Mnemonic functions served by the hippocampus are especially susceptible to age-related decline due to the vulnerability of this brain region to physiological insults (Foster, 1999). In humans and rodents, hippocampal lesions cause deficits in long-term memory or memory recall in a delay-dependent manner with concomitant preservation of short-term memory (Milner, 1972; Winocur, 1988). In aged humans and rodents, memory impairments with similar temporal loss-of-function are also prevalent (see Foster, 1999; Winocur, 1988). Long-term memory, unlike short-term memory, requires protein synthesis and gene transcription (Davis & Squire, 1984). The same is true for cellular correlates of memory such as long-term potentiation (LTP) (Bliss & Collingridge, 1993). LTP has an early protein synthesis-independent phase as well as a late protein synthesis-dependent phase (Krug, Lossner, & Ott, 1984) and it is the late phase, specifically, in which aged rats exhibit deficits (Barnes, 1979; Landfield, McGaugh, & Lynch, 1978).

Recent studies have identified relationships between proteins involved in signal transduction and spatial memory among aged rats that include kinases (Colombo & Gallagher, 2002; Colombo, Wetsel, & Gallagher, 1997), phosphatases (Foster, Sharrow, Masse, Norris, & Kumar, 2001), phospholipases (Nicolle, Colombo,

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Gallagher, & McKinney, 1999), and immediate early genes (Lanahan, Lyford, Stevenson, Worley, & Barnes, 1997; Yau, Olsson, Morris, Noble, & Seckl, 1996). It is thus likely that dysregulation in the expression or function of signal transduction molecules in hippocampal neurons is responsible for memory deficits among some aged subjects. Alterations in the levels or function of transcription factors may play important roles in age-related memory impairment due to the requirement for protein-synthesis in long-term memory formation.

Members of the cAMP-response element binding protein (CREB) family of transcription factors have been implicated in the formation of both long-term memory (Bartsch et al., 1995; Bourtchuladze et al., 1994; Dash, Hochner, & Kandel, 1990; Impey et al., 1998; Yin et al., 1994) and late-phase LTP (Impey et al., 1996; Schulz, Siemer, Krug, & Höllt, 1999). CREB is a constitutive transcription factor from a family of genes that share similar structural domains (i.e., basic region and leucine zipper) and contain both activators and repressors. CREB (referred to here as CREB1) is an activator of transcription, whereas CREB2 (also known as ATF4, mATF4, C/ATF, TAXREB67, and mTR67, for review see Hai & Hartman, 2001) has been shown to be both a transcriptional activator (Liang & Hai, 1997) as well as a transcriptional repressor (Karpinski, Morle, Huggenvik, Uhler, & Leiden, 1992).

CREB1α/Δ (CREB α and Δ are two isoforms of the CREB1 gene generated by alternative splicing) knockout mice reportedly show deficits in both spatial memory and protein synthesis-dependent late-LTP in the hippocampus (Bourtchuladze et al., 1994). In contrast, Graves, Dalvi, Lucki, Blendy, and Abel (2002) reported that CREB1α/Δ mutant mice had normal spatial learning in the water maze. Gass et al. (1998), however, reported that CREBcomp mice, in which the β isoform of CREB was disrupted in addition to the α and Δ isoforms, were impaired in water maze learning but exhibited intact hippocampal LTP. They suggest the deficit in water maze learning resulted from an inability to switch search strategies rather than a specific spatial memory deficit. In rodents, specific and temporally controlled inhibition of hippocampal CREB1 using antisense administration impairs long-term but not short-term spatial memory for the Morris water maze (Guzowski & McGaugh, 1997), whereas augmenting CREB1 levels in the basolateral amygdala through viral vector transfer specifically facilitates long-term memory for fear conditioning after a training regimen designed to produce only short-term memory (Josselyn et al., 2001).

The relationship between CREB2 and memory has been studied in non-mammalian systems only. An *Aplysia* homologue of CREB2, ApCREB2, was shown to inhibit CREB-mediated transcription in F9 cells. It was subsequently shown that antibodies to ApCREB2 were able to transform short-term facilitation into long-term facilitation (Bartsch et al., 1995), a state similar to mammalian long-term memory formation in that it requires both transcription and translation (Montarolo et al., 1986). It is not known, however, if CREB1 and CREB2 play similar opposing roles in mammalian long-term memory formation.

Investigation of the role of CREB in age-related memory impairment has begun only recently. Aged rats display decreased hippocampal CREB1–DNA binding (Asanuma et al., 1996). Foster et al. (2001) reported decreased phosphorylated CREB in the hippocampus of aged rats in comparison with young adult rats, whereas basal CREB1 levels did not differ between the two groups. In those studies, however, the relationship between basal CREB1 levels and memory among aged rats was not examined. Since aged rats exhibit a wide range of individual variability in mnemonic function, the current study examined the levels of hippocampal CREB1 and CREB2 among individual young and aged rats after spatial memory training.

2. Materials and methods

2.1. Subjects

Three- and 9-month-old male Long-Evans rats were obtained from Charles River Laboratories, Raleigh, NC. The rats were housed individually at Johns Hopkins University in wire mesh cages in a temperature-controlled room (25 °C) under a 12 h light (7 AM–7 PM)/dark cycle with ad libitum access to food and water. The place-learning task was conducted when the rats were 6- and 24-months old, respectively. One week following place training, the animals were shipped to Tulane University and housed under identical conditions. The rats were allowed to acclimate for one additional week before transfer training took place. All rats were screened at the time of sacrifice for viral antibodies and necropsies. Three aged rats were excluded from both the behavioral and biochemical analyses due to the presence of pituitary tumors. The remainder tested negative for viral antibodies.

2.2. Behavioral testing

2.2.1. Place learning

The rats were trained to find a fixed, hidden platform in a water maze (diameter 1.83 m × height 0.58 m) enclosed by a black curtain with cues attached to the inside. The pool water was clouded with ~250 ml white tempera paint and maintained at 27 °C. A white platform (34.5 cm high) was located 1 cm below the water surface. The rats received three trials/day for eight days. For each trial the rat was placed in one of four random start locations and allowed 90 s to find the platform. If the rat