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CELLULAR PRION PROTEIN MODULATES AGE-RELATED BEHAVIORAL AND NEUROCHEMICAL ALTERATIONS IN MICE

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Abstract—The cellular prion protein (PrP^C) is a neuronal-anchored glycoprotein that has been associated with various functions in the CNS such as synaptic plasticity, cognitive processes and neuroprotection. Here we investigated age-related behavioral and neurochemical alterations in wild-type ($Prnp^{+/+}$), PrP^C knockout ($Prnp^{0/0}$) and the PrP^C overexpressing Tg-20 mice. Three- or 11 month-old animals were submitted to a battery of behavioral tasks including open field, activity cages, elevated plus-maze, social recognition and inhibitory avoidance tasks. The 11 month-old $Prnp^{+/+}$ and $Prnp^{0/0}$ mice exhibited significant impairments in their locomotor activity and social recognition memory and increased anxiety-related responses. Remarkably, Tg-20 mice did not present these age-related impairments. The i.c.v. infusion of STI1 peptide 230–245, which includes the PrP^C binding site, improved the age-related social recognition deficits in $Prnp^{+/+}$. In comparison with the two other age-matched genotypes, the 11 month-old Tg-20 mice also exhibited reduced activity of seric acetylcholinesterase, increased expression of the protein synaptophysin and decreased caspase-3 positive-cells in the hippocampus. The present findings obtained with genetic and pharmacological approaches provide convincing evidence that PrP^C exerts a critical role in the age-related behavioral deficits in mice probably through adaptive mechanisms including apoptotic pathways and synaptic plasticity.

Key words: aging, cellular prion protein (PrP^C), behavior, acetylcholinesterase (AChE), synaptophysin, caspase-3.

Aging is often accompanied by a decline in several aspects of sensorimotor and cognitive functions. Behavioral changes also represent one of the important aspects of aging in humans and rodents, and for this reason they are consistently focuses of the gerontological literature (Griffiths et al., 1987). Several mechanisms have been sought regarding the neurochemical, structural and functional changes in the CNS during aging (for review see Lister and Barnes, 2009). For instance, age-related cognitive decline in both human and rodents are associated, at least in part, with degeneration of the basal forebrain cholinergic neurons that project to cortical areas and hippocampal formation (Bartus et al., 1982; Gallagher and Colombo, 1995). Moreover, there is increasing evidence indicating that dysfunction and loss of nerve terminals might represent one of the earliest modifications in the course of age-related deficits in memory function (Selkoe, 2002; Coleman et al., 2004; Lister and Barnes, 2009). Thus, compounds with different pharmacological profiles, such as acetylcholinesterase (AChE) inhibitors and presynaptic modulators, aimed at increasing central cholinergic neurotransmission and avoiding synaptic loss, respectively, have been suggested as potential drugs to counteract age-related cognitive decline (Coleman et al., 2004; Youdim and Buccafusco, 2005; Lister and Barnes, 2009).

Cellular prion protein (PrP^C) is a cell-surface glycosylphosphatidylinositol-anchored protein that is highly expressed in many brain regions including the hippocampus and cortical areas (Sales et al., 1998; Moleres and Velayos, 2005). Additionally, both presynapticaly and postsynapticaly PrP^C expression has been demonstrated (Sales et al., 1998). Mouse models in which the PrP^C gene (Prnp) was ablated have been used in several studies examining behavior and cognition. The first studies with PrP^C-knockout mice suggested that these animals have no gross anatomical abnormalities in the brain and visceral organs and that the deficiency of this cellular protein was not enough to cause significant behavioral abnormalities (Bueler et al., 1992; Manson et al., 1994). However, more recent studies have pointed some differences between PrP^C knockout mice and wild-type control group ($Prnp^{+/+}$) (Martins et al., 2002; Spudich et al., 2005; Coitinho et al., 2006; Weise et al., 2006; Lobao-Soares et al., 2007; Nazor et al., 2007; Xikota et al., 2008). PrP^C-null mice exhibit significant alterations in sleep, circadian rhythm (Tobler et al., 1996) and we have recently demonstrated that brain levels of PrP^C in mice can affect exploratory behaviors, anxiety, locomotor performance, equilibrium, as well as the time needed to adapt to new environments (Lobao-Soares et al., 2007). Studies investigating the cognitive performance of PrP^C-null mice have been inconsistent, with some authors demonstrating learning and memory impairments (Nishida et al., 1997; Criado et al., 2005), while

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Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; ANOVA, analysis of variance; DAB, 3,3′-diaminobenzidine; DG, dentate gyrus; DTNB, dithiobisnitrobenzoate; GSS, Gerstmann-Straüssler-Scheinker; LTM, long-term memory; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PD, Parkinson's disease; PKA, protein kinase A; Prnp, gene that codify PrP^C protein; PrP^C, cellular prion protein; STI1, stress-inducible protein; STM, short-term memory; TNB, 5-thio-2-nitro-benzoic acid.

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others have reported no significant differences (Bueler et al., 1992; Roesler et al., 1999; Xikota et al., 2008).

Of high interest, Coitinho et al. (2003) showed an early development of age-related cognitive deficits in PrP^C-null mice. This response seems to be associated, at least in part, to synaptic impairments since PrP^C is necessary for normal hippocampal synaptic functioning (Collinge et al., 1994). Moreover, long-term potentiation (LTP), which is a form of synaptic plasticity considered to be a cellular mechanism underlying learning and memory (Malenka and Nicoll, 1999), is significantly reduced in the CA1 region of the hippocampus in aged PrP^C-null mice (Curtis et al., 2003). Although these results were not reproduced in other studies (Maglio et al., 2006).

Additionally, previous findings suggest that PrP^C might confer neuroprotection against oxidative stress (Brown et al., 1997) and apoptotic cell death (Martins et al., 2002; Roucou and LeBlanc, 2005). PrP^C modulates the extent of neuronal loss after ischemic insults (Shyu et al., 2005; Spudich et al., 2005) and confers neuroprotection against seizures induced hyperexcitability and excitotoxicity (Walz et al., 1999). Moreover, a growing body of research has emphasized the participation of PrP^C in disorders associated with aging such as Alzheimer's disease (AD) (Ferrer et al., 2001; Aguzzi and Haass, 2003; Schwarze-Eicker et al., 2005) and Parkinson's disease (PD) (Wang et al., 2008).

Many of those functions rely on PrP^C binding to other proteins such as the co-chaperone stress-inducible protein (STI1) (Martins et al., 1997; Zanata et al., 2002). Remarkably, PrP^C interaction with either full-length STI1 or the STI1 peptide 230–245 (PepSTI1230–245), induced neuroprotective signals that rescued retinal neurons from apoptosis (Zanata et al., 2002), through a cAMP/PKA-dependent pathway (Chiarini et al., 2002). Moreover, STI1 is highly expressed in the hippocampus and induces neuritogenesis and neuroprotection in hippocampal neurons (Lopes et al., 2005). Interestingly, the interaction between PrP^C and STI1 also modulates short-term memory (STM) formation and long-term memory (LTM) consolidation in rats (Coitinho et al., 2007). Taken together, these early findings suggest that STI1 may be involved in various functions of PrP^C in the CNS including synaptic plasticity, cognitive processes and neuroprotection.

Therefore, the aim of the present study was to investigate the role of PrP^C in the behavioral and neurochemical alterations observed during aging in mice. Three- or 11-month-old wild-type ($Prnp^{+/+}$), PrP^C knockout ($Prnp^{0/0}$) and PrP^C overexpressing Tg-20 mice were submitted to a battery of behavioral tasks including the open field, activity chamber box, elevated plus-maze, social recognition and inhibitory avoidance tasks. Indeed, we investigated whether the interaction of STI1 with PrP^C affects short-term social memory of aging $Prnp^{+/+}$ mice. Finally, we performed quantification of seric AChE levels and determination of synaptophysin and caspase-3-cleaved immunoreactivity in the hippocampus, used as markers of synaptic density and apoptotic cell death, respectively. Collectively, the present results provide new molecular and functional insights into the role played by PrP^C in age-related changes in the CNS.

EXPERIMENTAL PROCEDURES

Animals

Male knockout mice homozygous for the disrupted *Prnp* gene, *Prnp* null mice (designated $Prnp^{0/0}$ mice) produced as previously described (Bueler et al., 1992), wild-type ($Prnp^{+/+}$) and PrP^C overexpressing Tg-20 male mice were donated by Dr. Vilma R. Martins from the Ludwig Institute for Cancer Research (Sao Paulo, Brazil) when they were 3 months old. The animals were maintained in the Animal House of the Federal University of Santa Catarina (Brazil) until the age of 11 months. Mice were maintained in groups of four–five animals per cage (42×34×17 cm) under controlled temperature (23±1 °C), with a 12 h light cycle (lights on 7:00 AM) and free access to food and water. The age of 11 month-old was selected because of the high mortality observed in $Prnp^{0/0}$ mice, and this fact has limited the investigation in advanced age points. All animals used in this study weighted 30–40 g.

The $Prnp^{0/0}$ mice used were descendants of Zrch I animals (Bueler et al., 1992), while the wild-type ($Prnp^{+/+}$) controls were generated by crossing F_1 descendants from a 129/Sv×C_{57}BL/6J mating. Tg-20 animals were obtained by insertion of extra copies of Prnp in blastocystis from $Prnp^{0/0}$ animals (Fischer et al., 1996). The genotype of the animals was confirmed by polymerase chain reaction (PCR) with DNA extracted from the tail, at an annealing temperature of 60 °C in 35 cycles, using the following primers: forward (5′-ATCAGTCATCATGGCGAAC-3′) and reverse (5′-AGAGAATTCTCAGCTGGATCTTCTCCCGTC-3′). A band of 693 bp corresponds to the *Prnp* sequence in the wild-type animals, while a band of 1635 bp represents the neomycin cassette, which replaced the *Prnp* sequence and thus identifies $Prnp^{0/0}$ mice. All procedures used in the present study complied with the guidelines on animal care of the local Ethics Committee on the Use of Animals (CEUA/UFSC) which follows the NIH publication "Principles of Laboratory Animal Care".

Drug treatment

$PepSTI1_{230-245}$ (ELGNDAYKKKDFDKAL), which represents the PrP^C binding site at the STI1 molecule, and a STI1 peptide not related to PrP^C, $PepSTI1_{422-437}$ (QLEPTFIKGYTRKAAA), were previously described (Zanata et al., 2002).

Biochemical approaches demonstrated that PrP^C interacts specifically and with high affinity to the co-chaperone STI1 (Zanata et al., 2002). Mapping experiments revealed that the binding sites are located in amino acids 113–128 in PrP^C and in amino acids 230–245 in STI1 (Zanata et al., 2002). The $PepSTI1_{422-437}$ is a randomic peptide also derived from STI1 but in contrast with the portion 230–245, the sequence 422–437 did not represent a binding site for PrP^C, in this way selected as the innocuous negative control.

The concentration of the both peptides (50 ng/μl) was defined as previously (Coitinho et al., 2007), and the peptides were diluted in sterile 0.1 M phosphate-buffered saline (PBS) (pH 7.4). The i.c.v. microinjections were performed using a microsyringe (5 μl, Hamilton Co., Reno, NV, USA) connected to a 26-gauge stainless-steel needle that was inserted perpendicularly 3 mm deep through the skull according to the procedure originally described by Haley and McCormick (1957) and modified by Laursen and Belknap (1986). Briefly, the animals were anesthetized with isoflurane 0.96% (0.75 CAM; Abbot Laboratórios do Brasil Ltda., RJ, Brazil) using a vaporizer system (SurgiVet Inc., WI, USA) and then gently restrained by hand for i.c.v. injections. The sterilization of the injection site was carried out using a gaze embedded in 70% ethanol. Under light anesthesia (i.e. just that necessary for loss of