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Neurobiology of Aging 28 (2007) 748–765

NEUROBIOLOGY OF AGING

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Changes in neuropeptide expression in mice infected with prions

Margarita Diez [a,*], Darlene Groth [b], Stephen J. DeArmond [b,c], Stanley B. Prusiner [b,d,e], Tomas Hökfelt [a]

[a] *Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm, Sweden*
[b] *Institute for Neurodegenerative Diseases, University of California, San Francisco, CA 94143, USA*
[c] *Department of Pathology, University of California, San Francisco, CA 94143, USA*
[d] *Department of Neurology, University of California, San Francisco, CA 94143, USA*
[e] *Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143, USA*

Received 1 July 2005; received in revised form 24 February 2006; accepted 28 February 2006
Available online 18 April 2006

Abstract

Prion diseases are neurodegenerative disorders characterized by accumulation of an aberrantly folded isoform (PrP^{Sc}) of the normal prion protein (PrP^{C}). Using in situ hybridization and immunohistochemistry, we have studied changes in the expression of neuropeptides, acetylcholinesterase and tyrosine hydroxylase in CD1 and FVB wild-type mouse strains, as well as in PrP^{C} null mice and in mice overexpressing PrP^{C} following intracerebral inoculation with RML or Me7 prions. In the immunohistochemical analysis, neuropeptide Y (NPY), enkephalin and dynorphin-like immunoreactivities increased in mossy fibers of CD1 and FVB mice inoculated with either RML- or Me7 prions, whereas cholecystokinin-like immunoreactivity was decreased. These changes in peptide levels were paralleled by an increase in the transcripts in granule cells for neuropeptide Y, enkephalin, and cholecystokinin. However, the dynorphin transcript was decreased in the granule cells. The changes occurred more rapidly in PrP^{C}-overexpressing compared to wild-type mice, and could not be found at all in PrP^{C}-knockout mice. These changes in peptide expression, which mostly occur before appearance of symptoms of disease, may reflect attempts to initiate protective and/or regenerative processes.

Keywords: CCK; Dentate gyrus; Enkephalin; Galanin; Hippocampus; Immunohistochemistry; In situ hybridization; Knockout; Neuropeptide Y; Scrapie; Transgenic mice

1. Introduction

Prion diseases include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker disease (GSS), fatal familial insomnia (FFI) and kuru in humans; bovine spongiform encephalopathy (BSE) of cattle; and scrapie of goats and sheep [87]. The central feature of prion diseases is the posttranslational conversion of the cellular isoform of the prion protein (PrP), designated PrP^{C} [79], to the abnormal, misfolded isoform, designated PrP^{Sc} [3,73], whereby the α-helical content of the protein is diminished and the amount of β-sheet is increased [82]. This conformational conversion renders PrP^{Sc} partially resistant to proteolytic degradation

Abbreviations: AChE, acetylcholine esterase; BSE, bovine spongiform encephalopathy; CA1–CA3, Ammon's horn fields CA1–CA3, respectively; CCK, cholecystokinin; DG, dentate gyrus; CJD, Creutzfeldt–Jakob disease; FFI, fatal familial insomnia; FITC, fluorescein isothiocyanate; GABA, γ-aminobutyric acid; GrDG, granule cell layer of the dentate gyrus; GSS, Gerstmann–Sträussler–Scheinker disease; IB, inner blade; -LI, -like immunoreactivity; Lmol, stratum lacunosum moleculare; Mol, stratum moleculare; NPY, neuropeptide tyrosine (Y); nt, nucleotide; OB, outer blade; OE, overexpressing; Or, oriens layer; PBS, phosphate-buffered saline; PoDG, polymorph layer of the dentate gyrus; *Prnp*, prion protein-encoding gene; Py, pyramidal cell layer; Rad, stratum radiatum; tg, transgenic; RML, mouse prion strain from Rocky Mountain Laboratory; TH, tyrosine hydroxylase; TSA, tyramide signal amplification; WT, wild type

* Corresponding author at: Department of Clinical Neuroscience, Neuroimmunology Unit, Karolinska Intitutet, 171 76 Stockholm, Sweden. Tel.: +46 8517 76561; fax: +46 8517 76248.
E-mail address: Margarita.Diez@cmm.ki.se (M. Diez).

0197-4580/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2006.02.017

[73,74,79,86,87]. In the cell, PrP^{Sc} has a long half-life, as it cannot be properly degraded, although recent studies imply that PrP^{Sc} can be degraded by macrophages [2] and dendritic cells [66].

PrP^{C} is encoded by the *PRNP* gene (*Prnp* in mouse) which is located on chromosome 20 in the human genome and the syntenic chromosome 2 in mouse [14]. PrP^{C} is found in sphingolipid- and cholesterol-rich plasma membrane domains referred to as lipid rafts or caveolae-like domains, where it is bound to the cell membrane by a glycosylphosphatidyl inositol anchor [5,50,94]. PrP^{C} is expressed in almost all cell types, but mainly in neurons [58], and is constitutively expressed in adult, uninfected brain [16,79]. In neurons, PrP^{C} is highly concentrated at the synapse [90] and has been shown to be expressed both pre- and postsynaptically [41,51,89].

Prion diseases are characterised by the accumulation of PrP^{Sc}, astrogliosis, activation of microglia cells and vacuolization of nerve cell processes resulting in the typical spongiform neurodegeneration [25]. However, vacuolization is not always observed in mouse models for prion-infection [31–33,88]. The mechanisms underlying neurodegeneration are largely unknown, but appear to be mediated by apoptosis induced by neurotoxicity, which has been demonstrated both in vitro [8,40,75,78] and in vivo [18,35,47,48,65,108]. Recent studies implicate the involvement of increased oxidative stress and neuronal apoptosis in prion-infected neuronal cells, both in the brains of patients with CJD and in brains of scrapie-infected mice [49,75]. Additionally, neuronal nitric oxide synthase (nNOS) is impaired in scrapie-infected mice [57,80] and localizes abnormally in PrP^{C}-knock-out (KO) mice [57].

After experimental transmission of scrapie from goats prions into rodents, scrapie strains [112] with distinct and stable properties could be isolated after infection in the same inbred mouse strain [11,28]. Different prion strains in mice are defined by the incubation time and pattern of neuropathology.

Since it has now been recognized that many neurodegenerative diseases show common features, in particular protein misfolding has been emphasized [77,93], we thought it relevant to compare prion mice with some Alzheimer mouse models, where we previously have reported marked changes in neuropeptide expression [30,34].

In the present study we have therefore explored: (i) to what extent prion infection causes changes in expression of a number of neuropeptides and two enzymes, (ii) if changes depend on host genetic background and/or prion strain and (iii) if changes in peptidergic expression precede the clinical symptoms. Thus, we studied the expression of several neuropeptides, with focus on neuropeptide tyrosine (NPY) [96], which has been shown to be particularly strongly regulated in a number of experimental models, including mouse Alzheimer models [30,34] and epilepsy/seizures [46,92], and to protect against neurodegenerative processes [100]. Moreover, several neuropeptides, such as galanin, have been shown to have trophic effects both in the periphery and the central nervous system [109], indicating that peptides may be upregulated to counteract neurodegeneration (see e.g. [21]). Finally, we studied the transmitter synthesizing/inactivating enzymes tyrosine hydroxylase (TH) and acetylcholinesterase (AChE). Wild-type (WT) mice, two strains, CD1 and FVB mice, transgenic mice devoid of PrP^{c} (PrP^{C}-KO), and transgenic mice overexpressing PrP^{C} (PrP^{C}-OE mice) were studied after infection with the RML or Me7 prion strain.

2. Materials and methods

2.1. Animals

Two-month-old male CD1 and FVB mice (Charles River Laboratories, Wilmington, MA) were inoculated intracerebrally with 30 μl of one of the following inocula: RML prions (isolated from passage from Cheviot sheep brain to Compton goats and thereafter to Chandler and CD1 mice) [15]; Me7 prions (from Suffolk sheep brain homogenate passaged into C57Bl mouse) [29]; or diluent containing 5% bovine serum albumin (BSA) (Sigma, St. Louis, MO) in phosphate-buffered saline (PBS). Mice were inoculated using a 27-gauge needle inserted into the right parietal lobe. PrP^{C}-KO mice ($Prnp^{0/0}$/94% FVB) and PrP^{C}-OE mice (Tg(MoPrP-A)B5043/$Prnp^{0/0}$ 94% FVB) [98] were inoculated in the same manner. Thus the following groups of mice were studied: (1) CD1 mice inoculated with RML prions, analysed at 60 and 120 days postinoculation (CD1 + RML); (2) FVB mice inoculated with RML prions, analysed 100 and 140 days postinoculation (FVB + RML); (3) CD1 and FVB mice inoculated with Me7 prions, analysed 110, 150 and 160 days postinoculation (CD1 + Me7 and FVB + Me7, respectively); (4) PrP^{C}-KO mice inoculated with RML prions, analysed at 110 and 140 days postinoculation (PrP^{C}-KO + RML); (5) PrP^{C}-OE mice inoculated with RML prions, analysed at 18, 30 and 45 days (PrP^{C}-OE + RML). All mice were generated, treated and sacrificed at UCSF (decapitation and freezing for in situ hybridization and picric acid–formalin perfusion for immunohistochemistry) and then shipped to Karolinska Institutet for histochemical analysis. Five mice per group and time point were used for, respectively, in situ hybridization and immunohistochemistry. Moreover, five control mice, sacrificed at the latest time point in each group, were also analysed. In order to attempt to minimize the number of mice in the experiments, we refrained from using saline controls. We considered this appropriate, since in three earlier studies on prion mice by our group using similar methodologies [31–33] we included the diluent saline as control at each time point, and no difference could be seen between groups.

2.2. In situ hybridization

Animals were killed by carbon dioxide followed by decapitation, their brains quickly removed and immersed in