Available online at www.sciencedirect.com

SCIENCE DIRECT

Brain Research 1056 (2005) 10 – 21

BRAIN RESEARCH

www.elsevier.com/locate/brainres

Research Report

Expression of PrP^C in the rat brain and characterization of a subset of cortical neurons

Francisco J. Moleres, José L. Velayos*

Department of Anatomy, Faculty of Medicine, University of Navarra, Irunlarrea s/n, 31080 Pamplona, Spain

Accepted 25 June 2005
Available online 16 August 2005

Abstract

The cellular prion protein (PrP^C) is a membrane-bound glycoprotein mainly present in the CNS. The scrapie prion protein (PrP^{Sc}) is an isoform of PrP^C, and it is responsible for transmissible spongiform encephalopathies (TSEs), a group of neurodegenerative diseases affecting both humans and animals. The presence of the cellular form is necessary for the establishment and further evolution of prion diseases. Here, we map the regional distribution of PrP^C in the rat brain and study the chemical nature of these immunopositive neurons. Our observations are congruent with retrograde transport of prions, as shown by the ubiquitous distribution of PrP^C throughout the rat brain, but especially in the damaged areas that send projections to primarily affected nuclei in fatal familial insomnia. On the other hand, the presence of the cellular isoform in a subset of GABAergic neurons containing calcium-binding proteins suggests that PrP^C plays a role in the metabolism of calcium. The lack of immunostaining in neurons ensheathed by perineuronal nets indicates that prions do not directly interact with components of these nets. The destruction of these nets is more likely to be the consequence of a factor needed for prions during the early stages of TSEs. This would cause destruction of these nets and death of the surrounded neurons. Our results support the view that destruction of this extracellular matrix is caused by the pathogenic effect of prions and not a primary event in TSEs.

Theme: Disorders of the nervous system
Topic: Degenerative disease (other)

Keywords: Calcium-binding protein; Perineuronal net; Prion protein; Transmissible spongiform encephalopathy; Wistar rat

1. Introduction

The cellular prion protein (PrP^C) is a 33- to 35-kDa membrane-bound glycoprotein attached to the cell surface by a glycosylphosphatidylinositol anchor [40,57]. In spite of being ubiquitously distributed throughout many tissues and cell types in most mammalian species, PrP^C is especially abundant in the central nervous system (CNS) [7,10,53]. The specific role of this protein in the CNS remains to be determined, although it has been involved in several functions such us cellular adhesion, neuroprotection and cell signalling [43].

PrP^{Sc}, also termed prion, is the *scrapie* or pathogenic isoform of PrP^C. This altered isoform is responsible for transmissible spongiform encephalopathies (TSEs), a group of fatal neurodegenerative diseases affecting both humans and animals [9]. The presence of PrP^C is necessary for the establishment and further evolution of these diseases [13]. PrP^{Sc} has been postulated to interact with the host's PrP^C [1], inducing the transformation of its α-helices into a β-sheet conformation and thus provoking the transformation of PrP^C into PrP^{Sc} [21,39,49].

The main neuropathological features of TSEs are spongiform change, neuronal loss and gliosis, as well as PrP^{Sc}

Abbreviations: A, anterior complex of the thalamus; CB, calbindin; CBP, calcium-binding proteins; CJD, Creutzfeldt–Jakob disease; CR, calretinin; FFI, fatal familial insomnia; MD, mediodorsal thalamic nucleus; PNN, perineuronal nets; PrP^C, cellular prion protein; PrP^{Sc}, scrapie prion protein; PV, parvalbumin; TSEs, transmissible spongiform encephalopathies

* Corresponding author. Fax: +34 948425649.
E-mail address: jvelayos@unav.es (J.L. Velayos).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.06.067

deposits [20,52]. TSEs can be classified into sporadic (Creutzfeldt–Jakob disease, sCJD), familial (familial CJD, fCJD; Gerstmann–Straüssler–Scheinker syndrome, GSS; fatal familial insomnia, FFI) and infectious (kuru; iatrogenic CJD, iCJD; new variant of CJD, vCJD). In animals, TSEs mainly affect sheep (*scrapie*) and cattle (bovine spongiform encephalopathies, BSE) [19].

The alteration of the GABAergic system in human and experimental TSEs has been consistently described [5,11,12,14,24–26,30–33,37,60]. Parvalbumin (PV), calbindin (CB) and calretinin (CR) are three types of calcium-binding proteins (CBP) expressed in non-overlapping populations of GABAergic cells within the cerebral cortex [15]. The PV-positive neurons are particularly affected in CJD, mainly those surrounded by perineuronal nets (PNN) [5,32].

Studies about the localization of PrP^C to date have always been limited due to the lack of specific antibodies directed against PrP-fixed epitopes. Although the localization of PrP^C has been previously described in hamsters and mice, neither the presence of PrP^C in brains of rat nor the neurochemical partners of PrP^C in vivo have been deeply analyzed.

The localization of PrP^C within the CNS is a key step to know the potential structures liable to be affected by the scrapie agent. Moreover, the study of the chemical nature of the PrP^C-containing neurons is fundamental to understand both the biology of PrP^C in vivo and the pathophysiology of PrP^{Sc} in human and experimental TSEs. The present study will also give new insights into the propagation mechanisms of PrP^{Sc} within the CNS in TSEs.

2. Materials and methods

The experiments were carried out in accordance with the guidelines of the European Communities Council Directive (86/609/EEC) and the Animal Care Committee of the University of Navarra. Great care was taken to avoid animal suffering.

2.1. Tissue processing

Five male Wistar rats weighing 250–300 g were anaesthetized with Ketolar (0.4 g/250 g) and decapitated for Western blot. Their encephala were rapidly removed, carefully dissected, placed in liquid nitrogen and stored at −80 °C until further use. Frozen tissue was homogenized and solubilized with 5 volumes of a mixture of proteases inhibitor (Roche, Basel, Switzerland) in RIPA (10 mM Tris, pH7.4; 150 mM NaCl; 1% Triton X-100; 1% deoxycholate; 0.1% SDS; 5 mM EDTA) (proportion 1:8). Samples were centrifuged at 13,000 × *g* at 4 °C for at least 15 min. Supernatant was separated and the content of proteins was measured with BSA.

Fifteen age- and sex-matched Wistar rats were used for immunohistochemistry. Animals were anaesthetized with chloral hydrate (1 ml/100 g) and transcardially perfused with Ringer's saline rinsing solution in distilled water to clean blood from vessels. Brains were removed, fixed for 24 h in 10% formalin at room temperature and embedded in paraffin. Brains were then sagittally ($n = 10$) or coronally ($n = 5$) cut at 3 μm.

2.2. Antisera

A monoclonal (6H4, Prionics, Zurich, Switzerland) and a polyclonal (Rb anti-HuPrP, Assay Dessigns, Ann Arbor, MI, USA) were used to detect PrP in Western blot and immunohistochemistry. Although labelling gave a similar pattern with both antisera, 6H4 yielded better results in immunoblotting experiments whereas the polyclonal gave very satisfactory results in immunohistochemistry, as recently described [42].

2.3. Western blot

20 μg of total protein was electrophoresed through a 12% SDS–PAGE gel. Proteins were transferred to a PVDF membrane. After blocking with 10% non-fatted milk in TBST, membranes were incubated with a monoclonal against the C-terminal region of PrP (6H4, Prionics, Zurich, Switzerland) at 1:7500 in TBST and the signal was developed by enhanced chemiluminescence (Amersham Biosciences, Buckinghamshire, England). Quantification was performed using Scion Image software.

Immunoblotting experiments were reproduced at least twice on each animal ($n = 5$).

2.4. Immunohistochemistry

Tissue was deparaffinized at 60 °C for 30 min and rehydrated from xylene to decreasing gradients of ethanol. The endogenous peroxidase was inactivated at 3% H_2O_2 in H_2O_d for 9 min. In order to retrieve tissue antigenicity, slides were then microwaved for 30 min in 0.01 M citrate buffer (pH 6.0) and cold at room temperature, followed by incubation with 20% normal serum in TBS for 30 min at RT to block non-specific binding.

2.4.1. PrP immunohistochemistry

A polyclonal antibody produced in rabbit (Assay Dessigns, Ann Arbor, MI, USA) recognizing the N-terminal region of PrP was added at 1:100 in TBS–1% BSA and incubated o/n at 4 °C. Sections were then incubated with biotynilated anti-rabbit IgG (1:200; Chemicon, Temecula, CA) for 30 min at room temperature, followed by incubation in an avidin–biotin complex solution (ABC, Vector Laboratories, Burlingame, CA) at 1:100 in TBS for 30 min at room temperature. Development of the signal was performed with 3,3′-diaminobenzidine (Sigma, St. Louis, MO, USA). Slides were dehydrated with graded ethanol series, and then passed through pure xylene. Some sections