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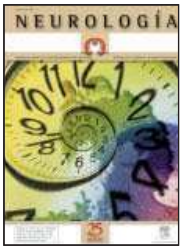

ORIGINAL ARTICLE

Cellular prion protein in the central nervous system of mammals. Anatomoclinical associations

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KEYWORDS
Cellular prion protein;
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Abstract
Introduction: The scrapie prion protein (PrPsc) requieres the celular prion protein (PrPc) for its propagation and replication. In this work we studied the expression and localization of the PrPc in the central nervous system (SNC) of the rat, mouse, cat, cow and human, using immunohistochemistry and Western blot techniques to understand more about prinopathies and Alzheimer's disease (EA).
Material and methods: For the immunohistochemistry study we used human, cat, rat and cow samples to analyse frontal, temporal and occipital cortex, as well as the hippocampus and the thalamus. For the Western blot analysis we used mouse, cat, cow and human brain samples.
Results: We observed a decrease in the amount of PrPc in the central nervous system (CNS) in a rostrocaudal shift in the species mentioned above. We observed inhibitory cells in the cat cortex. The Western blot analysis showed a similar pattern of expression in the different species studied with a preponderance of the diglycosylated band, in relation to the other bands observed in the analysis.
Discussion: These data suggest that in prionopathies PrPsc could be transmitted and could be replicated in and from the areas with most expression of PrPc. Similarly, a higher amount of this protein (PrPc) in some brain areas could explain some histopathological aspects of Alzheimer's disease (AD).
Conclusions: Our findings support the hypothesis of a retrograde transport of PrPsc in the CNS. PrPc could be related to the pathophysiology of AD.

PALABRAS CLAVE
Proteína priónica celular;

La proteína priónica celular en el sistema nervioso central de mamíferos. Correlatos anatomoclínicos

Resumen
Introducción: La proteína priónica celular patógena (PrPsc) necesita de la presencia de la fisiológica (PrPc) para su propagación y replicación. Se estudia comparativamente la ex-

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Prionopatías;
Enfermedad
de Alzheimer;
Mamíferos

presión y localización de PrPc en el sistema nervioso central (SNC) de rata, ratón, gato, vaca y humano, mediante técnicas inmunohistoquímicas y de *Western blot*, con el objetivo de un mejor conocimiento de las prionopatías y de la enfermedad de Alzheimer (EA).
Material y métodos: Se emplearon encéfalos humanos y de gato, rata y vaca, para estudios por técnicas inmunohistoquímicas; se analizaron las cortezas frontal, temporal y occipital, así como hipocampo y tálamo. Se utilizaron técnicas de *Western blot* para encéfalos de ratón, gato, vaca y humano.
Resultados: Existe una disminución rostrocaudal de la cuantía de PrPc en el SNC de dichas especies. PrPc se sitúa en la membrana y en el citoplasma de las neuronas. Se observan neuronas inhibitorias en el córtex del gato. El patrón general del *Western blot* es análogo en las especies estudiadas, con predominio de la banda diglucosilada sobre las bandas monoglucosilada y no glucosilada.
Discusión: Los datos indican que en las prionopatías, PrPsc puede transmitirse y replicarse de forma retrógrada en y a partir de las zonas más PrP positivas. La mayor cuantía de PrPc en algunas zonas del encéfalo humano podría estar en relación con los hallazgos anatomopatológicos de la EA.
Conclusiones: Los datos apoyan un transporte retrógrado de la PrPsc en el SNC. La PrPc debe de tener relación con la fisiopatología de la EA.

Introduction

Cellular prion protein (PrPc) is a membrane glycoprotein that forms part of various organs in most animal species studied. It is especially abundant in the central nervous system (CNS)[1].

There is a different isoform of this protein: the scrapie prion protein (PrPsc or prion, anagram of *PROteinaceous INfectious particle*). Prions are the causative agents of transmissible spongiform encephalopathies (TSEs), for whose establishment and subsequent evolution, the presence of PrPc along with PrPsc is required. In fact, it has been observed that knockout mice for PrP did not develop the disease when inoculated with PrPsc[2]. Both PrP isoforms are encoded by the same gene and present the same amino acid sequence, although with differences in their secondary structure. PrPc can be digested by proteinase K, while PrPsc is only partially digested.

PrPsc appears only in prionopathies, and reaches brain concentrations 10-20 times higher than PrPc. There are sometimes accumulations of PrPsc in the form of plaques in TSEs.

The most common form of TSE in humans is Creutzfeldt-Jakob disease (CJD). Severe or fatal familial insomnia (FFI) shows similarities to CJD. However, what is characteristic of FFI are the initial pathological changes of the midline of the dorsomedial thalamic nucleus (DM) and of the anteroventral nucleus of the complex of the anterior thalamic nuclei (AN)[3-5]. Interestingly, the mentioned thalamic areas have a different connectivity with respect to other DM and AN portions, as well as with respect to neighbouring thalamic nuclei to DM and AN, in both cats and rats[6,7]. After a time, in FFI cases with longer duration, other structures become affected, including the deep layers of the cerebral cortex[3,8]. The most affected cortical zones are the frontal and cingulate cortices, and the least, the occipital[8,9].

We have proposed the existence of a retrograde propagation of PrPsc from the DM and AN to various CNS areas in FFI[6,10]. Similarly, in other prionopathies, the propagation must be retrograde (for example, in bovine spongiform encephalopathy[11]). Moreover, in the rat CNS we have observed the co-localisation of PrPc with several neurotransmitters[7], which suggests a multiplicity of pathogenic mechanisms in both FFI and in TSEs in general.

There is also another special circumstance: we have observed a specific PrPc behaviour in brains with Alzheimer's disease (AD)[12].

This study aims to determine whether PrPc, both in prionopathy and in AD, must have a special role in relation to the pathophysiology of both groups of clinical entities.

Material and methods

We used human brains as well as those from the cat, rat, mouse and cow.

The brains of cat, rat, cow and human were studied by immunohistochemistry techniques, with a special focus on the analysis of the frontal, temporal and occipital cortices, the hippocampus and the thalamus.

The tissue was fixed by immersion, to obtain a correct antigen protection. It was included in paraffin and cut into 4Ìm sections. The best results were from polyclonal antibodies, Anti-Prion Protein 91511 (Assay Designs Inc.) and ARP-01-8634 (American Research Products).

We also carried out Western blot techniques with fresh tissue, not fixed, which was dissected into areas of interest for this purpose; samples were quickly frozen with dry ice and stored at -80°C until the quantification of the total protein content in each of the study areas. Subsequently, proteins were separated on 12% acrylamide gels and transferred to nitrocellulose membrane, with an incubation in which the antibody for PrP mAb 6H4 (Prionics) was used. Once the right conditions were obtained, we proceeded to

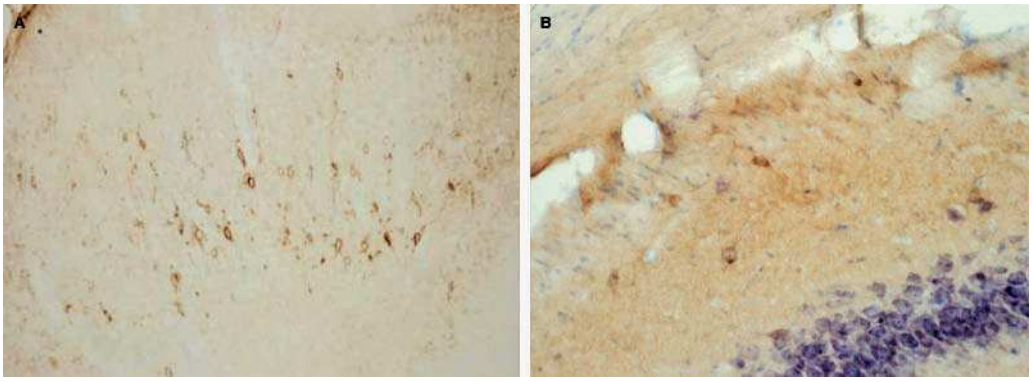

Figure 1 PrPc in rat frontal cortex (A) and hippocampus (B). Contrast was applied with haematoxylin-eosin for the hippocampus.

the corresponding measurement by densitometry. Cat, mouse, cow and human brains were used.

For the experiments we used a human brain from a 28-year old donor, considered healthy by the BCH in Navarra. The extraction was carried out within 6 hours of death after an accident.

The study was conducted in accordance with the 1975 Helsinki Declaration and approved by the Ethics Committee of the Faculty of Medicine at the University of Navarra.

Results

Immunohistochemistry (rat, cat, cow and human)

In the species studied (including humans), the amount of PrPc decreased caudally within the CNS (for example, comparing the thalamus and the cerebral cortex [figs. 1-4]).

It can be clearly observed that PrPc was located in the cell membrane (fig. 4) (human temporal cortex) and also in the cytoplasm (in this case, in the form of clusters; cow inferior olivary nucleus) (fig. 3).

It should be noted that in the cerebral cortex of all species, the deeper layers were more abundantly marked than the surface layers, and that there were GABAergic cells distributed throughout the CNS (for example, in cat occipital cortex) (fig. 2). This is a subject that was already studied by Moleres et al.[7,13] in rats.

Western blot

The Western blot was studied comparatively for frontal, temporal and occipital cortices, as well as for the thalamus in cat, cow and human brains (fig. 5).

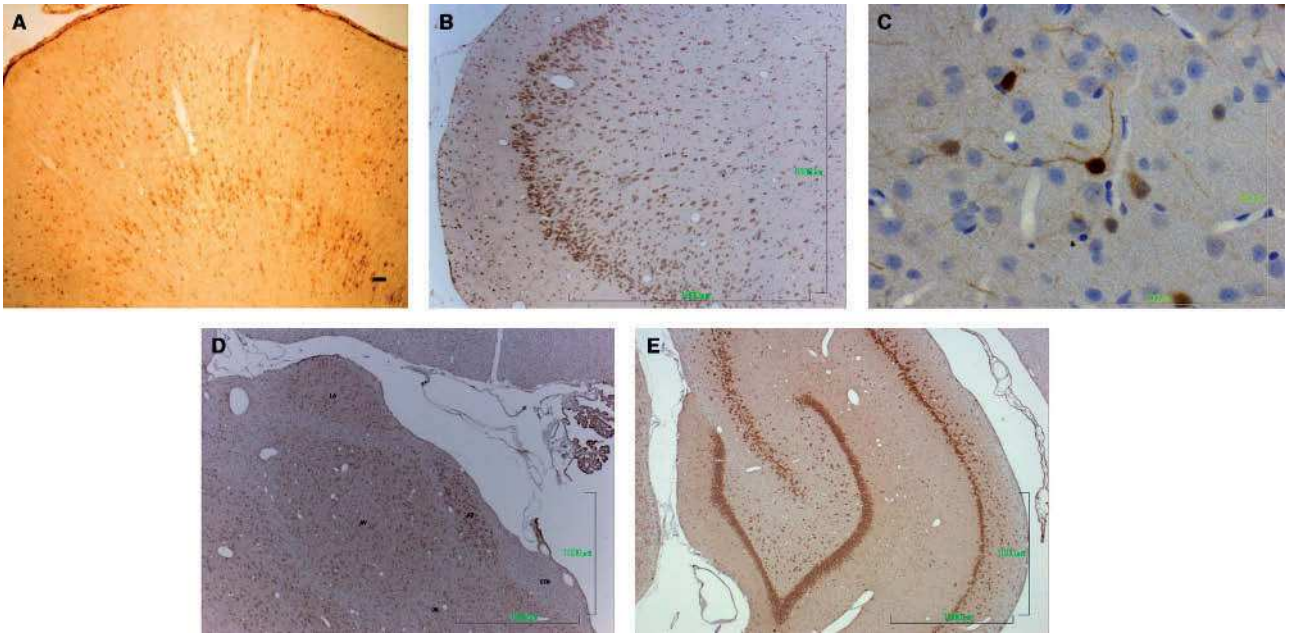

Figure 2 PrPc in cat frontal (A) and temporal (B) cortices and immunoreactivity to calbindin in the occipital cortex (C), as well as PrPc in the thalamus (D) and hippocampus (E). AD: anterodorsal nucleus; AV: anteroventral nucleus; DM: dorsomedial nucleus; LA: lateral anterior; STM: stria medullaris.

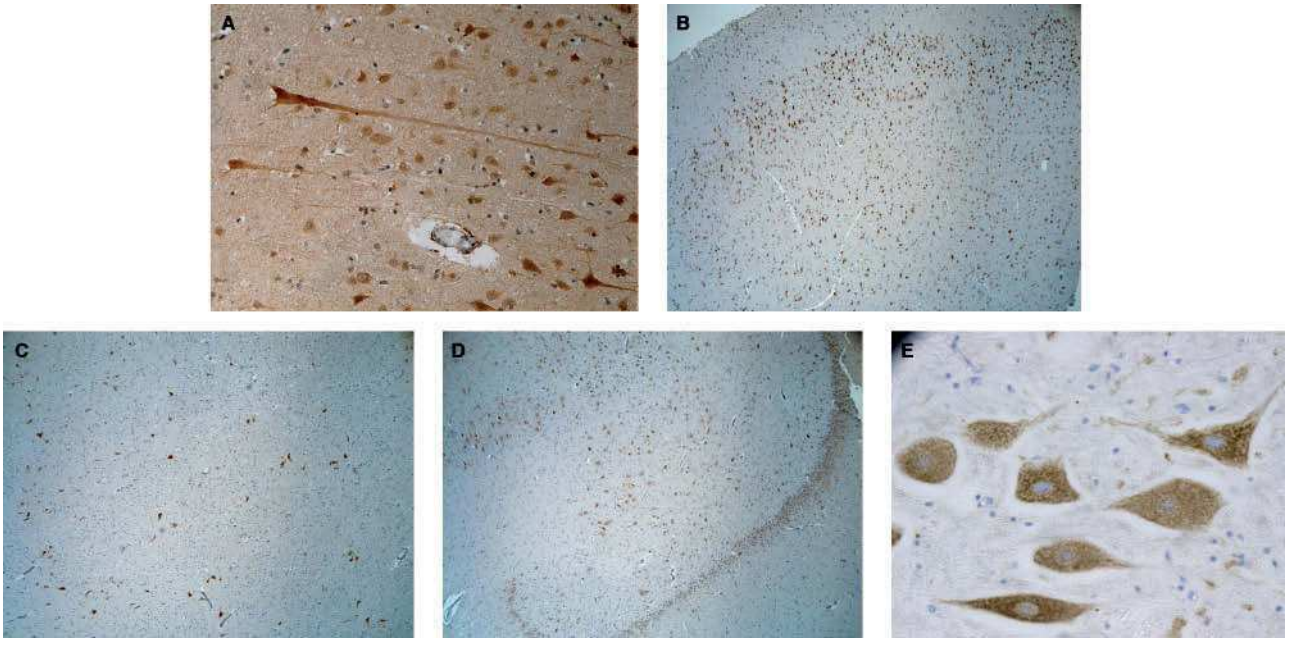

Figure 3 PrPc in cow frontal (A) and occipital (B) cortices and in the thalamus (C), hippocampus (D) and inferior olivary nucleus (E).

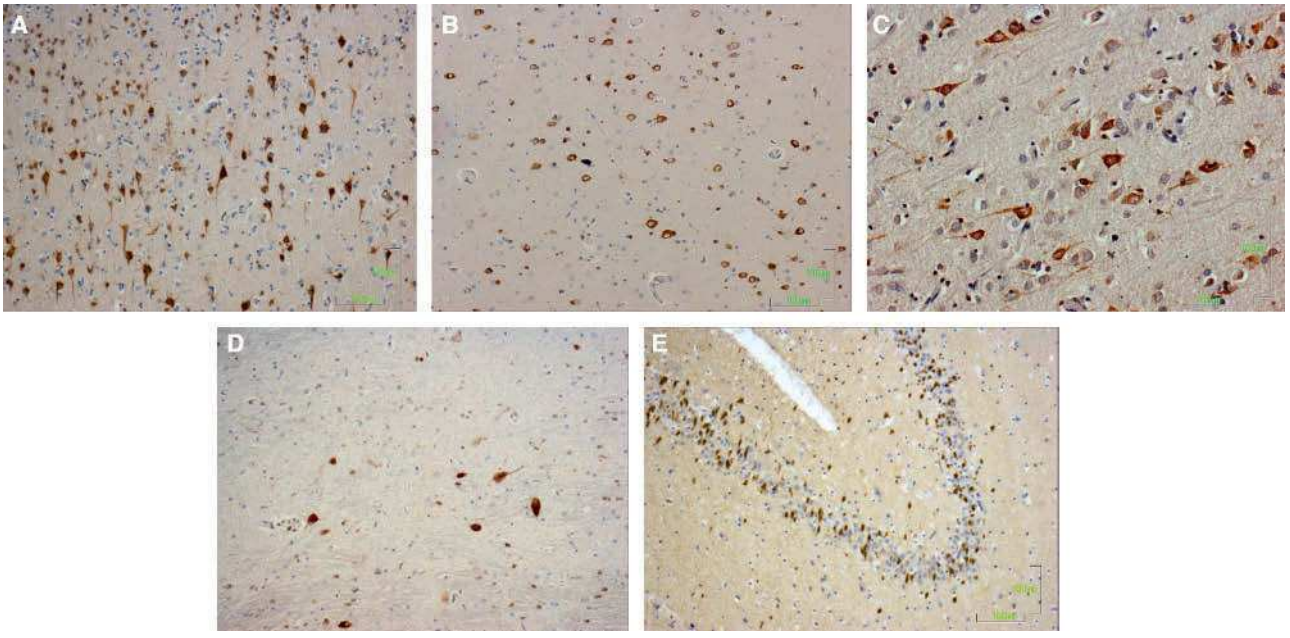

Figure 4 PrPc in human frontal (A), temporal (B) and occipital (C) cortices and in the thalamus (D) and hippocampus (E). Contrast was applied with haematoxylin-eosin for the hippocampus.

The amount of PrPc was higher in the cow than in the other species, except as regards the temporal cortex, where the amount was higher in humans. In all cases, the intensity was greater for the diglycosylated band than for the other bands corresponding to the protein (monoglycosylated and non-glycosylated) (fig. 5).

Figure 5C shows the comparative analysis of mouse, cat, cow and human samples with regards to the hippocampus. The amount of PrPc in cows was still more abundant, followed by the human, and the lowest amounts were for the cat and mouse. In all cases, the intensity of the diglycosylated band was greater than for the other bands.

Discussion

This is a continuation of our previous work[6,7,11-13].

With regard to the immunohistochemistry data, as in previous work, we continue to observe great ubiquity in the CNS, but with more abundance of protein in the more rostral

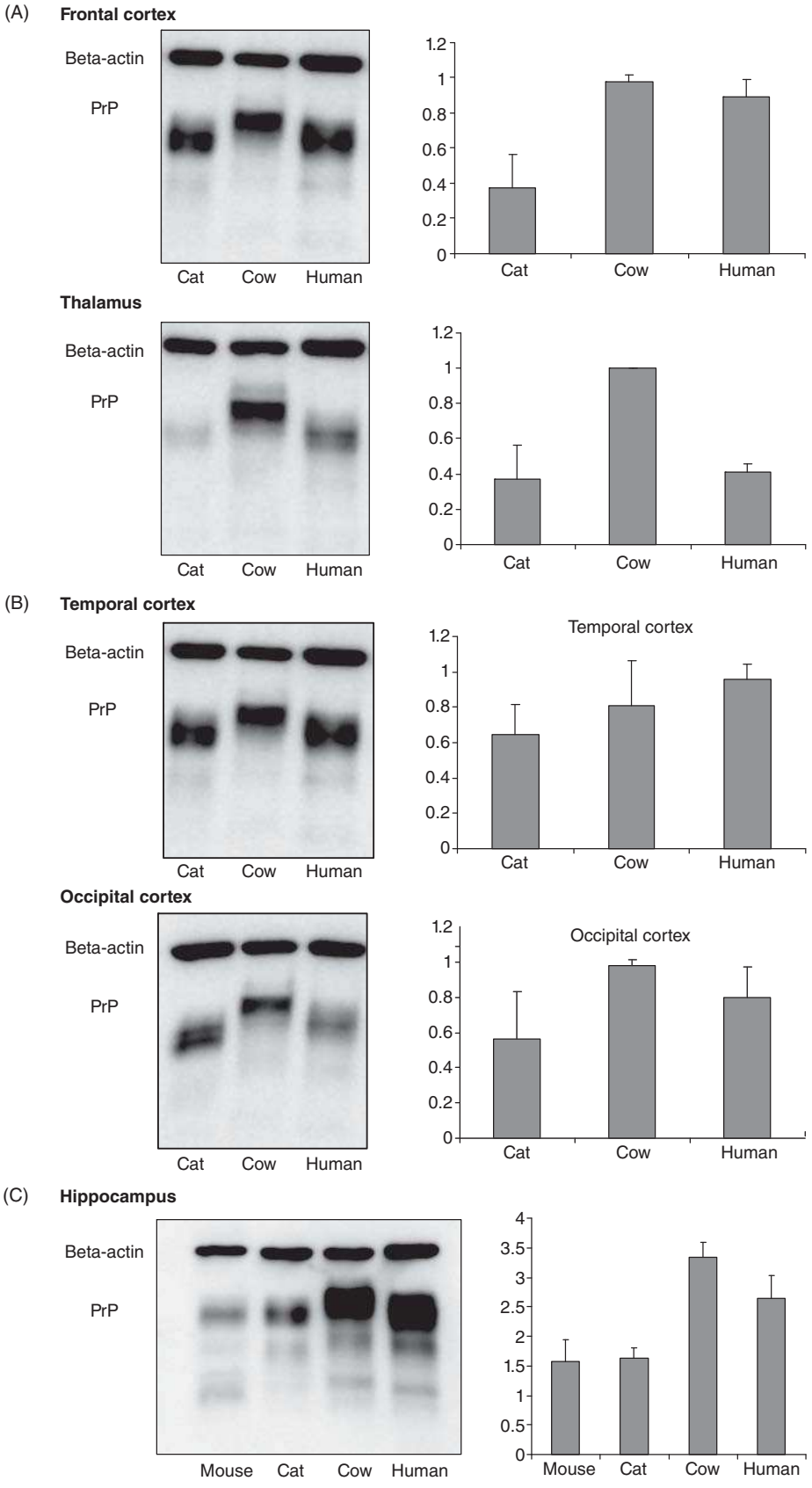

Figure 5 Comparative Western blots of cat, cow and human samples, for frontal cortex and thalamus (A) and temporal and occipital cortices (B). C: The analysis is of the hippocampus of mouse, cat, cow and human. Note the similar pattern in all three species, with increased intensity for the diglycosylated band with respect to the other two bands.

areas. This could mean that PrPsc replicates more intensely in and from these areas in prionopathies. The fact that in the species studied the amount of PrPc is especially abundant in areas that project towards the thalamus (such as the deep layers of the cerebral cortex [figs. 1-4]) indicates that the transmission of prions in TSEs (for example, in feline spongiform encephalopathy, bovine spongiform encephalopathy, human fatal familial insomnia) must follow a retrograde pathway within the brain[14,15]. Moreover, the occipital cortex projects less towards the thalamus, and from those considered, we can see in our study that this is the cortex containing less PrPc marking. This also supports the hypothesis of retrograde prion transport.

We observed that PrPc was located in both the cell membrane (fig. 1) and in the interior of neuronal cytoplasm (fig. 3).

There are no GABA cells in the cerebral cortex (fig. 2) (cat occipital cortex), as is also the case in rats[7,13]. This once again explains (by legitimate extrapolation) the fact that severe familial insomnia shows excitatory-type signs and symptoms, due to the destruction of inhibitory cells.

As for the data obtained from Western blots of some areas of the cerebral cortex, it seems logical that the amount of PrP in the temporal cortex should be more abundant in humans than in the other species studied. With respect to the hippocampus, it is interesting that the amount is higher in cows than in other species. The Western blot also shows a lower amount of PrP in the occipital cortex than in the other cortices considered, especially in humans, which is consistent with the immunohistochemical findings.

These data agree with the findings of Rezai et al.[16], who observed that the expression of PrPc was higher in the frontal cortex than in the occipital, both in healthy cases and in those of AD. It could also mean that the greater abundance of PrPc in certain cortical areas relates to the anatomopathological lesions of AD; such lesions are greater in the hippocampus, temporal cortex and frontal cortex than in other cortical CNS areas. This would not necessarily mean that PrPc was directly involved in the process of AD, as postulated by some authors[17]; it is simply a fact that we observe. However, we believe that PrPc would have a protective role in relation to AD[12], in accordance with recent studies by other authors[18-20].

In all cases, the diglycosylated band is more intense than the monoglycosylated and non-glycosylated bands. This situation is altered in cases of AD, where the amount of PrP diminishes and there is some intensification of the non-glycosylated band[12].

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