Evidence for the existence of a prion protein receptor

by

Russell Paul Bowler

A Thesis

Submitted in partial satisfaction of the
requirement for the M.D. with Thesis Program

of the
University of California, San Francisco

Abstract

Prion diseases are transmissable neurodegenerative disorders characterized by spongiform degeneration of gray matter, nerve cell loss, and amyloid plaque formation. A prion protein exists in normal brain and an abnormal form is found in prion diseases. Preliminary studies have indicated that a prion protein can bind to several brain proteins on Western blots. We attempted to characterize a putative prion protein receptor. Prion rods, radiolabeled with ^{125}I, were put into detergent-lipid-complexes and incubated with sections of adult and neonatal Syrian hamster brain. The most intense binding was seen in the region of the hippocampus with an IC_{50} of 2.8 nM. Binding was not displaced by agents which label the acetylcholine receptor. Prion rods were also added to primary neonatal Syrian hamster astrocyte cultures and the number of cells incorporating bromodeoxyuridine (BrdU) were counted. Addition of 0.1 µg/ml of prion rods caused a 45% increase in the BrdU labeling index over compared to cells not exposed to prion rods. These results suggest the presence of a prion protein receptor, but do not conclusively prove it.

Table of Contents

Introduction

This thesis will attempt to show that there exists a prion protein receptor relevant to the pathogenesis of scrapie disorders. A brief background of prion protein chemistry and several experiments which suggest the existence of a receptor for the prion protein will be presented.

Background

Prion disorders are primary neurodegenerative diseases characterized by spongiform degeneration of gray matter, nerve cell loss, and amyloid plaque formation. The feature which distinguishes these disorders from other neurodegenerative diseases is their transmissability. Examples of prion diseases include: infectious forms such as Kuru, iatrogenic Creutzfeld-Jakob disease (CJD) and scrapie in sheep; inherited forms such as Gerstmann-Sträussler-Schenker syndrome (GSS) and familial CJD; and sporadic forms of prion diseases which do not appear to have either an infectious or genetic etiology, such as CJD, which is the most common prion disease in humans. Considerable evidence suggests that a single brain protein, the prion protein (PrP), is central to both the etiology and pathogenesis of these disorders.

Because of the long incubation time associated with prion diseases, it was initially proposed that a slow virus was the etiologic agent. While small nucleic acid fragments have been found in highly purified infectious scrapie agent preparations, these are unlikely to represent the infectious genome [1, 2]. Instead extensive evidence points to the infectious agent being composed exclusively of an abnormal form of PrP. The term "prion" refers to a proteinaceous

infectious pathogen in order to distinguish the agents in these disorders from viruses and viroids [3].

Both a cellular isoform of the prion protein (PrP^C) and an infectious isoform in scrapie (PrP^{Sc}) are known to exist [4]. PrP^{Sc} is distinguished from PrP^C by its resistance to protease digestion, insolubility after detergent extraction, accumulation in secondary lysosomes, post-translational synthesis, and enrichment during purification of infectivity [3, 5-10]. The majority of PrP^C is attached to the outer cell membranes of neural cells by a phospho-inosital lipid side chain [11], but it can also exist as an integral membrane protein or can be secreted [12]. There is considerable evidence that PrP^{Sc} synthesis involves post-translational conversion of PrP^C to PrP^{Sc} [5, 13-15]. This conversion is initiated by prions. Nascent PrP^{Sc} forms into new prions which perpetuate the transformation of more PrP^C into PrP^{Sc}.

While the exact functions of PrP^C and PrP^{Sc} remain unknown, there is considerable evidence to suggest the importance of PrP^{Sc} in scrapie pathogenesis. It is known that PrP^{Sc} accumulates in extracellular amyloid plaques as a result of extracellular release of PrP^{Sc} [16]. Several studies suggest that local accumulation of PrP^{Sc} in the gray matter is causally related to the spongiform degeneration of neurons, reactive astrocytic gliosis, and amyloid plaque formation [16-18]. When PrP^{Sc} is injected into the thalamus of Syrian hamsters, the pattern of spread is as follows: synthesis of PrP^{Sc} begins unilaterally at the site of injection in the thalamus after one week; three weeks later accumulation is seen in the septum, suggesting spread by CSF rather than by neuroanatomical pathways;

also at this time disease spreads to the cerebral cortex [17-20]. Because spread of disease is discontinuous in the thalamus and not via known neuroanatomical pathways, it is possible that prions, released into the CSF, target specific nerve cell populations.

Several lines of evidence suggest that PrP^{Sc} may mediate an interaction between neurons and astrocytes in prion diseases [21]. CNS neurons synthesize most and perhaps all of both PrP^{C} and PrP^{Sc} [17]. Amyloid plaques composed of extracellular accumulations of filaments containing PrP^{Sc} demonstrates that PrP^{Sc} is released into the extracellular space [16]. Reactive astrocytic gliosis in Syrian hamster scrapie occurs precisely in brain regions containing PrP amyloid plaques and in regions of the neuropil where PrP^{Sc} accumulates. Finally, reactive astrocytic gliosis follows the accumulation of PrP^{Sc} within a brain region by 1-2 weeks[18].

A major challenge to the hypothesis that PrP^{Sc} is the sole functional component of prions is the existence of multiple prion strains or isolates [22]. Over 15 different scrapie prion isolates have been identified in mice and hamsters. Each is defined by a specific scrapie incubation time, distribution of spongiform degeneration, and whether or not amyloid plaques form [23, 24]. These characteristics are maintained unchanged during passages of a single isolate in an inbred mouse strain; however, they change significantly, or scrapie may even fail to occur, if the isolate is passaged into a different species. The latter observation has been termed the "host species barrier" and joins incubation time and neuropathology as a differentiating characteristic of prion isolates.

Another challenge is to explain how this information is coded in PrP^{Sc} and how it can be transferred to PrP^{C} during its conversion to PrP^{Sc}. Two possibilities exist: 1) There is a single form of PrP^{C} synthesized by each cell in the host and the infectious prion induces multiple stable structural changes in it during its conversion to PrP^{Sc}; Alternatively, 2) each neuron may synthesize a different form of PrP^{C}, each of which have the same amino acid sequence but differ in their carbohydrate trees or glycolipid anchor. For the latter possibility, one would postulate that the PrP^{Sc} in the infecting prion binds only homologous PrP^{C} during its conversion. Two lines of evidence support the latter. In transgenic (Tg) mice which express both mouse (Mo) PrP^{C} and Syrian hamster (SHa) PrP^{C}, hamster adapted prions were found to bind selectively to the SHa PrP^{C} and to convert it to SHa PrP^{Sc} whereas mouse adapted prions selectively reacted with Mo PrP^{C} and not SHa PrP^{C} [25, 26]. This argues that PrP^{Sc} targets PrP^{C} in a selective manner. The second piece of evidence comes from recent studies showing production and accumulation of PrP^{Sc} in different nerve cell populations for different prion isolates target [27].

The question then arises as to what mechanism could cause each prion isolate to target a different set of neurons to form PrP^{Sc}. One possibility is that each neuron synthesizes a different PrP^{C}. Another possibility is that each neuron has a different PrP^{Sc} receptor. A third possibility is that PrP^{Sc} binding to PrP^{C} on the surface of neurons requires a specific PrP^{C}-receptor complex.

Search for the prion protein receptor

Preliminary studies suggest that there are brain proteins which bind prion protein and may be a candidates for a receptor. Oesch et al. have shown that PrP 27-30, a 27-30 kDa proteinase K digestion product of PrP^{Sc} present in purified prion preparations, can be placed into detergent-lipid-protein-complexes (DLPC) which bind to glial fibrillary acidic protein (GFAP) and an unidentified 110 kDa protein on Western blots [28]. The significance of this binding is unknown. These studies were done with whole brain preparations and therefore lack regional specificity. Efforts to identify additional proteins binding to the prion protein by chemical cross linking have been unsuccessful (unpublished data). The function of both the prion protein and its putative receptor remain unknown, although it has been suggested that the prion protein mediates a neuron glial interaction.

Here we present results from tissue binding assays with PrP^{Sc} and Syrian hamster brains. We also report experimental evidence for a role of the prion protein, namely, causing glial cell proliferation. These are two lines of evidence which suggest the existence of a prion protein receptor.

Materials and Methods

Materials: Transfer membranes were purchased from BioRad, Richmond, CA (nitrocellulose, 0.45μm). $Na^{125}I$ (carrier-free; Amersham, Arlington Heights, IL) and iodobeads (Pierce, Rockford, IL) were used for radioiodination. Egg L-α-lecithin was obtained from Avanti-Polar, Pelham, AL. Bromodeoxyuridine (BrdU) was obtained from Ben Venue Laboratories Bedford, Ohio.

Histoblots: Animals were sacrificed by asphyxiation with CO_2. The brain was removed and frozen in powdered dry ice. Ten μm thick cryostat sections were cut, mounted on glass, thawed and pressed onto nitrocellulose membrane wetted in lysis buffer (0.5% Nonidet P-4, 0.5% sodium deoxycholate, 100 mM NaCl, 10 mM EDTA, 10 mM Tris-HCl, pH 7.8). The slide was pressed for 25 seconds and then checked for complete transfer of the section [19].

Ligand Blots: Non-specific binding to membranes was blocked by incubation in (20mM Tris-HCl, pH 7.5, 150mM NaCl, and 0.05% Tween 20)/5% blotto (nonfat dry milk) TBST . As probes, purified PrP 27-30 [29, 30] or PrP^C (immunoaffinity-purified on monoclonal anti-PrP antibody column as describe for PrP^{Sc} [8]) were radioiodinated to a specific activity of (2-5) X 10^7 cpm/μg. Unincorporated iodine was separated by precipitation of PrP with 9 volumes of absolute ethanol. For solubilization, labeled PrP was reconstituted into detergent-lipid-protein complexes (DLPC) (1 X DLPC: 20 mM Tris-HCL, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 1 mg/ml L-α-lecithin) at 0.1-0.5 μg of PrP/ml by sonication for 20 minutes. Binding of probes to histoblots was in TBST/0.5% blotto at (1-5) X 10^4 cpm/mL for 12-16 hours at room

temperature. Blots were washed in TBST/0.01 X DLPC for 2-4 hours, dried, and exposed to Kodak hyperfilm at -70° C.

Quantification of PrP^{Sc} binding: An estimate of the regional concentration of bound $Na^{125}I$-PrP 27-30 was made by measuring the average density using video assisted morphometric analysis. The histoblot image was captured using a PULNiX TM-745E high resolution CCD video camera which was interfaced with a BioQuant System IV image analysis system (R&M Biometrics, Nashville, TN). The average pixel density was measured by this system. The mean binding in the region of the stratum pyramidale of Ammon's horn in the hippocampus was determined by obtaining the average of 40 consecutive densitometric measurements of the autoradiographic signal. Background measurements were made over the stratum radiatum of Ammon's horn because of its uniformly weak binding signal. Background was subtracted from the stratum pyramidale measurements.

Primary astrocyte cultures: Neonatal Syrian hamster were sacrificed by overdose with pentobarbital. Astrocyte cell culture preparation was according to the method of Dichter[31] and Eng et al[32]. In brief, astrocytes were grown to subconfluence in culture medium containing fetal calf serum (FCS). FCS was removed overnight and then the "starved" cultures were exposed to either medium without FCS (control), medium plus 0.1 μg/ml PrP 27-30, medium plus FCS, or medium plus FCS and 0.1 μg/ml PrP 27-30 for 24 hours. The cultures were then incubated with bromodeoxyuridine (BrdU) for 5 hours, fixed in alcohol, and immunostained with anti-BrdU monoclonal antibodies. The

proportion of cells which incorporated BrdU was measured (figure 1). Approximately 1500 cells were counted for each experimental condition and each experiment was repeated three separate times.

Results

Binding in the normal adult Syrian hamster brain:

In the normal adult Syrian hamster brain, the most intense autoradiographic signals were found in the immediate region of the cell bodies of granule cell neurons in the dentate gyrus and pyramidal cell neurons in Ammon's horn of the hippocampus (Figure 2). Intense signal was also found in the medial habenular nucleus. Less binding occurred in the cerebral cortex, thalamus and hypothalamus. There was very little binding to white matter tracks. Previous studies have shown that PrP^C is absent from the medial habenular nucleus, the pyramidal cell layer of Ammon's horn, and the granule cell layer to the dentate gyrus [19]. This suggests that PrP 27-30 binds to regions of brain which have a low concentration of PrP^C.

Binding in the normal neonatal Syrian hamster brain:

Because the neonatal Syrian hamster brain has little PrP gene expression [17], it is a useful model to study binding of PrP 27-30 in the absence of PrP^C. We observed intense binding in the hippocampus and throughout the neocortex with little binding in the thalamus (figure 3). This suggests that the binding sites are not PrP^C, since PrP gene expression is not yet present. If more PrP binding sites are available because of a paucity of PrP^C, one might expect a more fulminant course of scrapie disease. This is consistent with the observation of rapid induction and wider distribution of scrapie disease when neonatal hamsters are inoculated [33].

Distribution of PrP 27-30 changes as animals become clinically sick with scrapie:

Normally clinical signs of scrapie occur about 60 days after Syrian hamsters have been inoculated with Sc237 prions. PrP^{Sc} begins to become detectable in the hippocampus after 35 days and then continues to rise sharply until death [18]. In scrapie infected hamsters, PrP 27-30 binding to the hippocampus was identical to the normal animal at 35 days post-inoculation, but little binding occurred at 65 days when the animals were clinically sick (figure 4). The inverse relationship between the amount of PrP^{Sc} present and the amount of PrP 27-30 binding suggests that PrP^{Sc} occupies available PrP binding sites as scrapie disease progresses.

Kinetics of PrP 27-30 - tissue interaction:

The observation that PrP 27-30 binding diminishes with the presence of PrP^{C} or PrP^{Sc}, suggests that there may be a common structure which binds PrP^{C}, PrP^{Sc}, and PrP 27-30. Using a competitive binding assay of labeled PrP 27-30 (4 nM) and unlabeled PrP 27-30 (0.25-80 nM), we determined the binding of PrP to the region of the stratum pyramidale of Ammon's horn in the hippocampus of the adult Syrian hamster. The normalized data from three assays produced a curve which showed that 70% of bound radiolabeled PrP 27-30 was displaced by about 30 nM unlabeled PrP 27-30 (figure 5). The remaining 30% was not displaced by further increases in the concentration of unlabeled PrP 27-30. The concentration of unlabeled PrP 27-30 that displaced 50% of the binding of labeled PrP 27-30 (IC_{50}) was 2.8 nM. A limited supply of PrP 27-30 precluded study at micromolar concentrations.

Binding of PrP is distinct from CNS cholinergic receptors:

Two lines of evidence raised the possibility that PrP binds to cholinergic receptors. The first is the observation that the Acetylcholine receptor-inducing activity (ARIA) protein, a chicken brain protein which upregulates acetylcholine receptors in cultured mammalian myocytes [34], has an amino acid sequence 30% homologous to mammalian prion proteins [35]. The second is that we found PrP binding to be anatomically similar to the distribution of nicotinic acetylcholine receptors. We tested this hypothesis by attempting to block PrP binding with α-bungarotoxin, lophotoxin, and monoclonal antibody to the nicotinic receptor. None of these blocked the PrP 27-30 binding. These observations suggest that the putative PrP receptor may be in the vicinity of the nicotinic acetylcholine receptor, but it is distinct from it.

PrP 27-30 rods cause gliosis and proliferation in primary astrocyte cultures:

The results of the tissue binding experiments and competitive binding assays provided indirect evidence for a PrP receptor. Proof of a receptor, however, requires showing correlation of binding with biologic activity. Although the function of PrP is unknown, histologic evidence suggests that PrP may be a mediator between neurons and glial cells.

The reactive astrocytic gliosis in prion diseases is often extremely intense, so much so that it has been termed "hypergliotic". Although gliofibrillogensis in scrapie has recently been quanitated by measurement of glial fibrillary acid protein (GFAP), the amount of glial proliferation has not [18].

In order to test the hypothesis that the prion protein causes glial proliferation, we added PrP 27-30 rods directly to primary neonatal Syrian hamster astrocyte cultures . The PrP 27-30 rods stimulated a 45% increase in the BrdU labeling index relative to the controls (figure 6). This increase was similar to that caused by the addition of FCS alone. The effect of adding PrP 27-30 and FCS were additive, yielding an approximately 108% increase in labeling index. After 10 days of continuous exposure to both PrP 27-30 and FCS, there was a doubling of the number of astrocytes relative to controls. Denaturing the PrP 27-30 by boiling it in sodium dodecylsulfate eliminated the effect on astrocyte proliferation. There was no effect in gliofibrillogenesis since the concentration of GFAP per cell remained unchanged.

Discussion

While it is relatively easy to show ligand binding, there is no equivalence between a binding site and a receptor site. In order to prove that there is a prion receptor, one must systematically show that the characteristics of a receptor are present. Laduron [36] suggests the following criteria be used: 1) drug displacement in the nanomolar range with agonists and antagonists belonging to a different chemical and pharmacological classes; 2) correlation between drug affinity in vitro and pharmacological potency in vivo; 3) regional distribution or tissue specificity; 4) subcellular distribution; 5) stereospecificity; 6) saturability; 7) reversibility; and 8) high affinity binding. Of these eight criteria, the first two, drug displacement and correlation with biological activity are the most important. Binding sites which do not fulfill these criteria are more likely to be "acceptor" sites and often lead to false interpretation of the importance of a binding protein. In reviewing our experimental results in the context of these criteria, the existence of a prion protein receptor is suggested but not proved.

Strong binding occurred at concentrations as low as 4 nM in the hippocampus of the adult and in the neocortex and hippocampus in neonates, showing that the binding is of high affinity. This binding was reversible. We were not able to prove that the binding was saturable because of limited quantities of the PrP^{Sc}. Regional selectivity or highly localized binding was seen in the hippocampus and medial habenular nucleus in the adult brain and in the neocortex and hippocampus in neonates.

Subcellular distribution was suggested by Oesch et al. [28]. On Western blots PrP 27-30 binds to intermediate filament proteins such as GFAP, vimentin, and the low molecular weight neurofilament protein in cytoskeletal preparations. In contrast, we did not see PrP 27-30 bind to any of these proteins in frozen section histoblots of both normal and scrapie infected adult and neonatal hamster brain. In particular, one would expect to have seen binding to white matter tracts which have high concentrations of neurofilaments and astrocytes which contain GFAP glial filaments. Whether or not the 110 kDa protein described by Oesch et al. [28] corresponds to the pattern and distribution of PrP 27-30 binding we observed remains undetermined.

Ligand displacement curves show that radiolabeled PrP 27-30 are displaced by nanomolar concentrations of unlabeled PrP 27-30 (IC_{50} = 2.8 nM). In general, displacement which occurs in the nanomolar range suggests ligand-receptor interaction, while displacement in the micromolar range is more characteristic of non-specific binding. In this study there was not enough ligand available to determine if complete displacement occurred with micromolar concentrations (30% of labeled bound ligand remained at the highest concentration of unlabeled ligand - 80 nM), therefore we could not eliminate the possibility of some further non-specific binding. Despite evidence that the putative prion receptor may reside in the region of the nicotinic acetylcholine receptor, we were unable to displace binding with agents which label acetylcholine receptors. Currently there are no known agents which displace prions.

The correlation of ligand binding with biologic activity has been more difficult to prove. There is indirect evidence for an effect on both glial and nerve cells. Histologically there is nerve cell loss, plaque formation, and gliosis. It has been shown that the degree of in vivo reactive astrocytic gliosis correlates directly with the degree of nerve cell loss in CJD [37]. We were able to identify an in vitro role for the prion protein: prion rods directly cause glial proliferation in cell culture independent of FCS. Further experiments will be necessary to show that this response correlates with prion affinity to its putative receptor.

We have shown that high affinity binding occurs in tissue specific locations in the Syrian hamster. This binding occurs in physiologic ranges and can be displaced. We have also shown that PrP 27-30 can directly cause glial proliferation. These observations suggest the existence of a prion receptor, but leave many questions unanswered. Does high affinity binding occur in other regions of the brain and does this binding have the same characteristics in the neonatal brain? Do different isolates of prions have different binding patterns? How does the prion protein enter cells? Are there subcellular prion receptors? What is the significance of the 110 kDa protein? Are there agents, perhaps astrocyte growth factors, which can displace prion binding? Finally is there a dose response relationship between prion rods and astrocyte proliferation and are there other physiologic responses to prions which can be measured? These are areas which need to be investigated in order to prove the existence of a receptor.

The significance of a PrP receptor remains enigmatic. Several general observations in this study lead us to suspect the importance of a receptor in both normal development and pathogenesis of scrapie disease. In normal neonatal hamsters there is little PrP^C synthesized until the brain becomes mature. As the brain matures there is more PrP^C and fewer available prion binding sites. One possible explanation for the difference of binding in the neonate is that, as the PrP^C is synthesized, it occupies the available binding sites, and limits the number available for exogenous PrP^{Sc}. The concept of a limited number of binding sites is also supported by observing the clinical progression of scrapie disease. PrP 27-30 binding is identical to normal uninfected adult Syrian hamsters before they become clinically ill, but there is little or no PrP 27-30 binding in the usual regions in hamsters which are clinically sick and near death from scrapie.

The possibility that PrP^{Sc} binds to sites normally maintained relatively unoccupied PrP^C raises several questions relevant to understanding the roles of PrP^{Sc} and PrP^C in the pathogenesis of scrapie. If there is a single molecule or receptor distributed to all brain regions which can bind PrP^{Sc}, PrP^C and PrP 27-30, then there must be mechanisms which determine how much PrP^C is distributed to each region containing the receptor. Alternatively, there may be multiple PrP-binding structures which have different affinities for PrP^C. Relevant to the pathogenesis of scrapie, the accumulation of PrP^{Sc} at sites normally maintained free of prion protein may be one mechanism leading to disease.

Acknowledgments:

This work was supported by research grants from the department of Pathology, University of California, San Francisco.

Tables and Figures

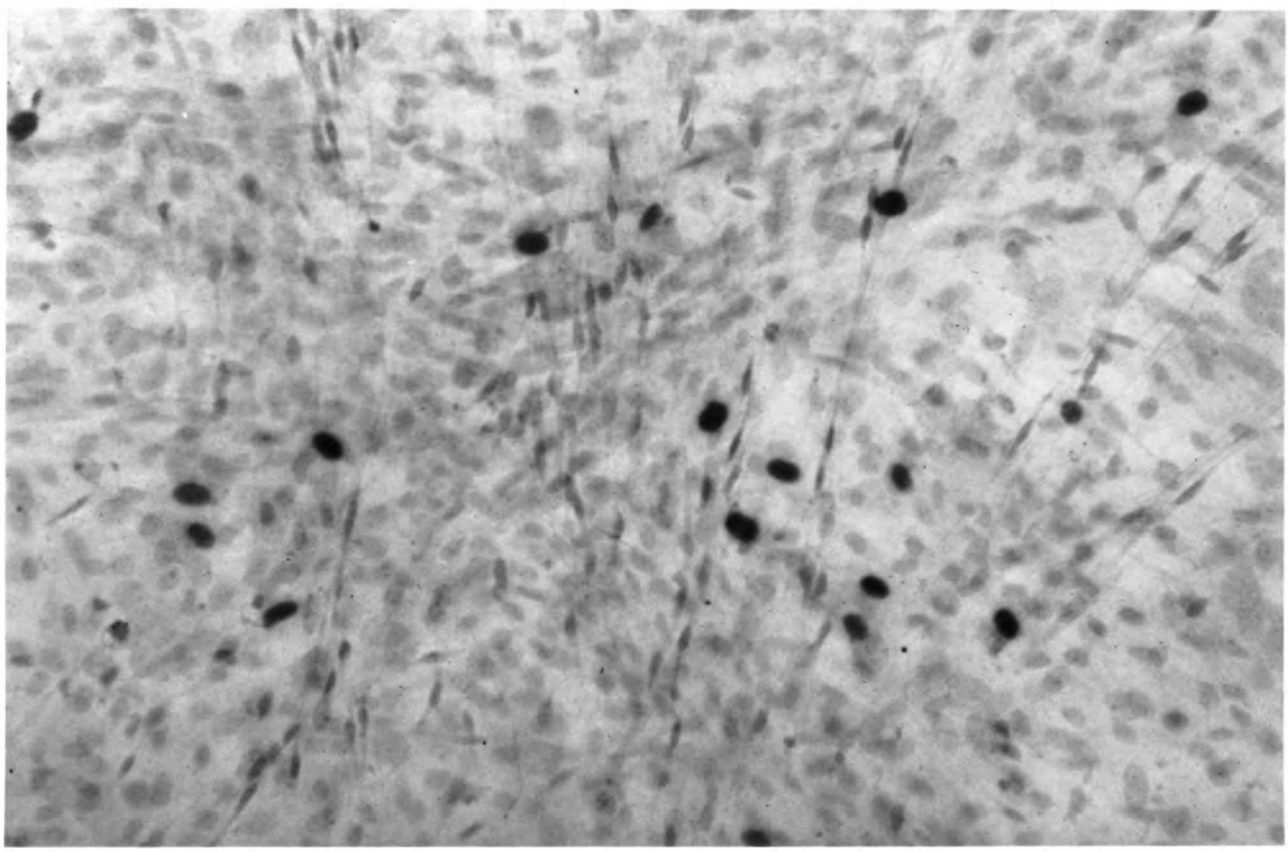

Figure #1: BrdU labeling of cultured neonatal astrocytes synthesizing DNA. Labeled nuclei are black.

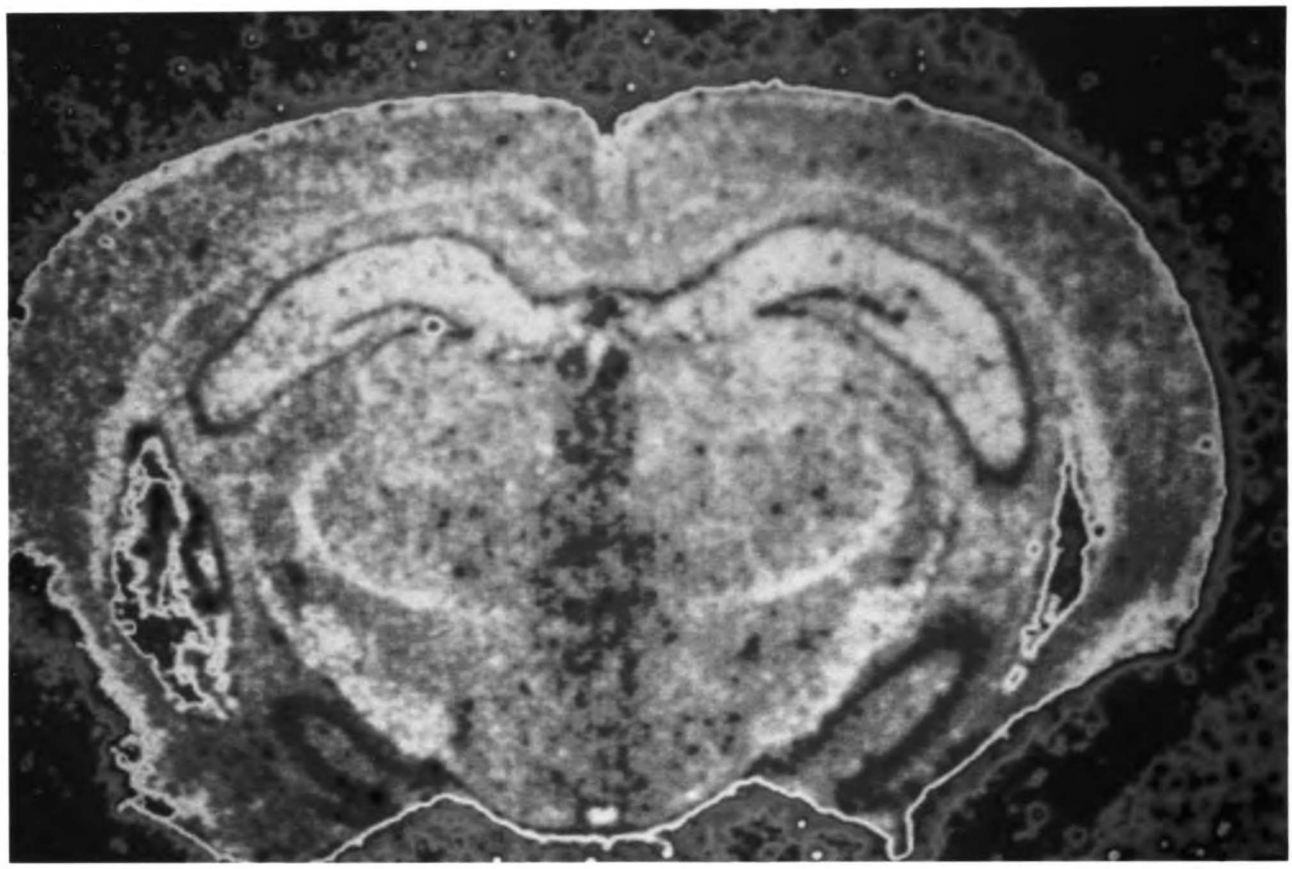

Figure #2: Binding of ^{125}I-PrP 27-30 DLPC to normal adult Syrian hamster brain. Areas of intense binding (hippocampus) are dark and areas of less intense binding are light.

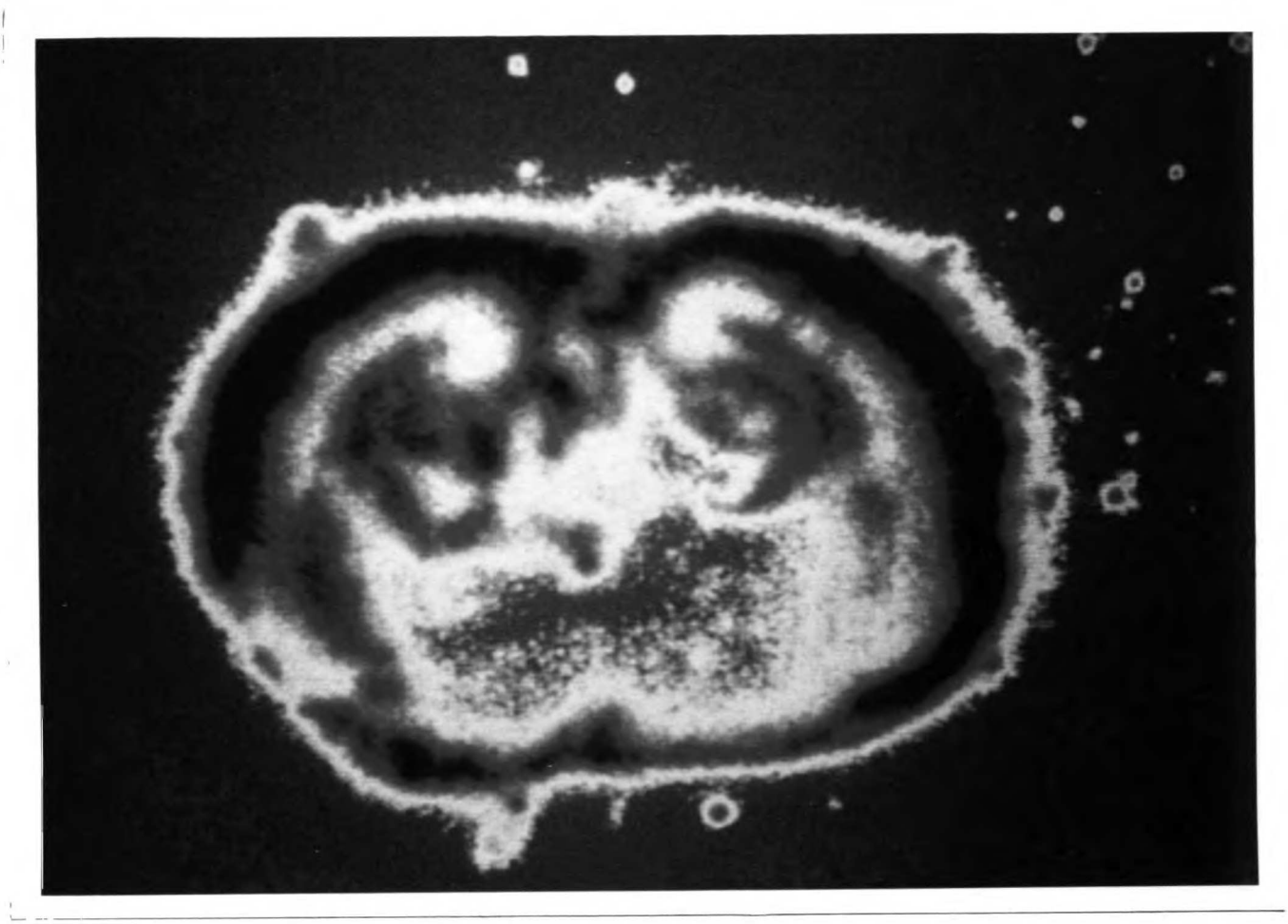

Figure #3: Binding of ^{125}I-PrP 27-30 DLPC to normal neonate Syrian hamster brain. Areas of intense binding are dark and areas of less intense binding are light.

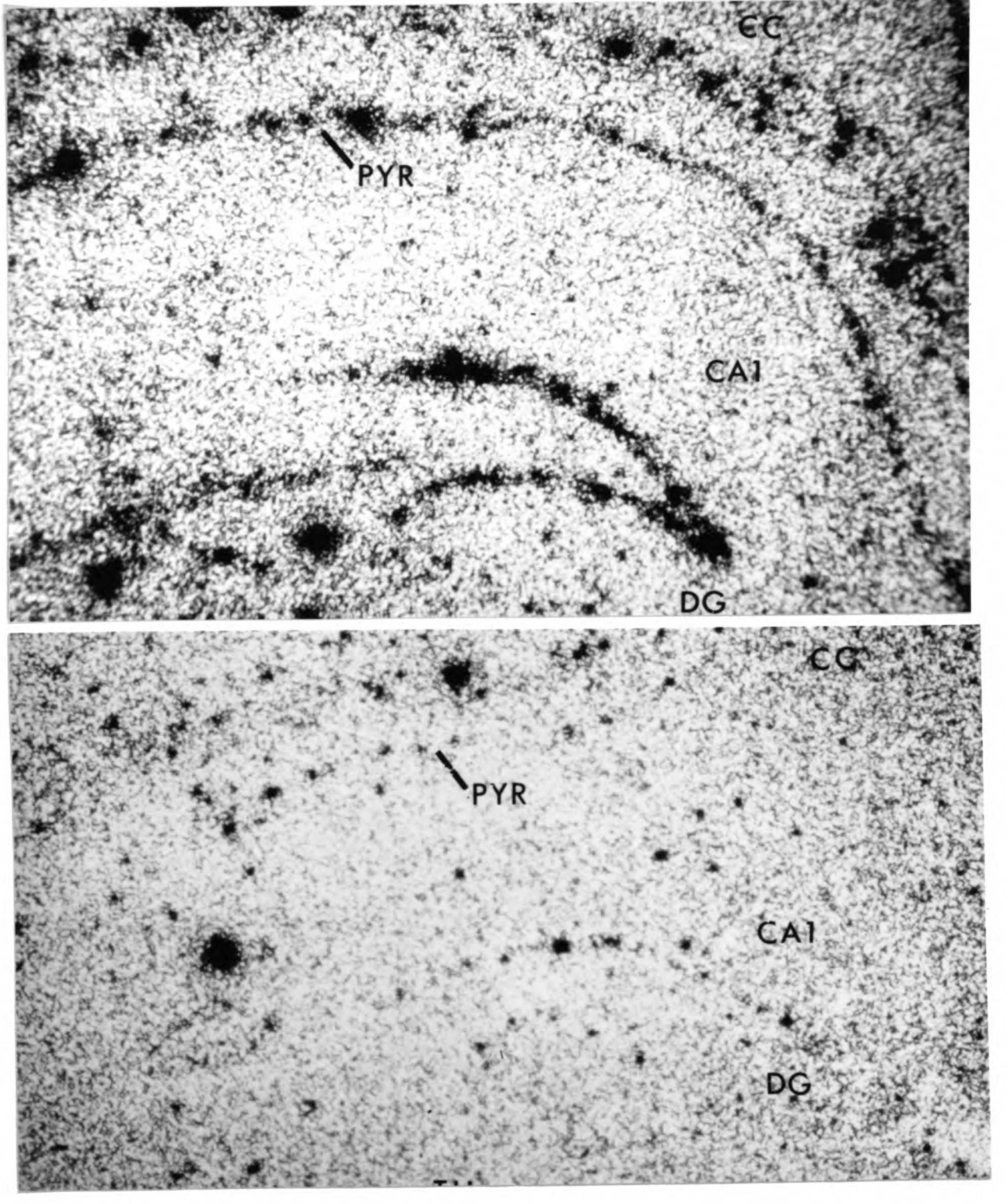

Figure #4: Binding of ^{125}I-PrP 27-30 DLPC to adult Syrian hamster brain in the region of the hippocampus at 35 (above) and 65 (below) days post PrP^{Sc} inoculation. Areas of binding appear dark.

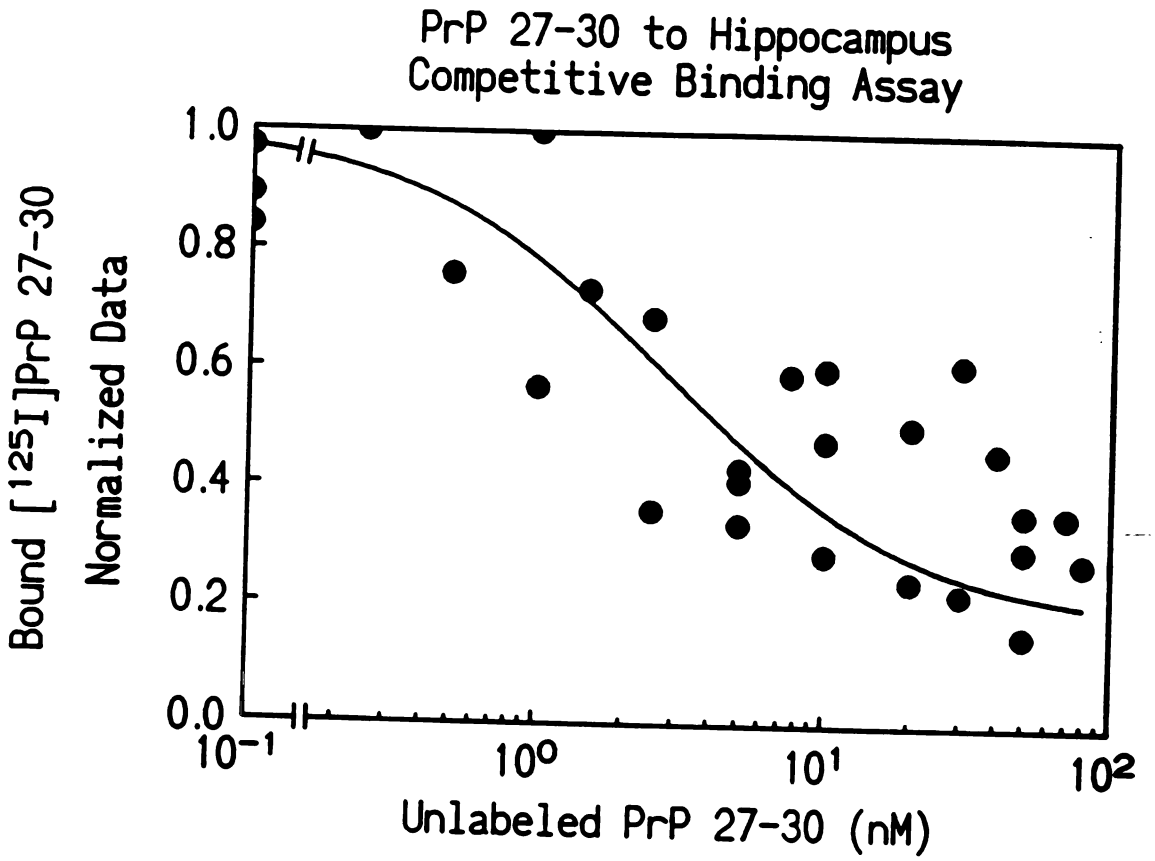

Figure #5: Competitive binding assay with ^{125}I-PrP 27-30 4.0 nM DLPC and varying concentrations of unlabeled PrP 27-30 DLPC on normal adult Syrian hamster brain histoblots.

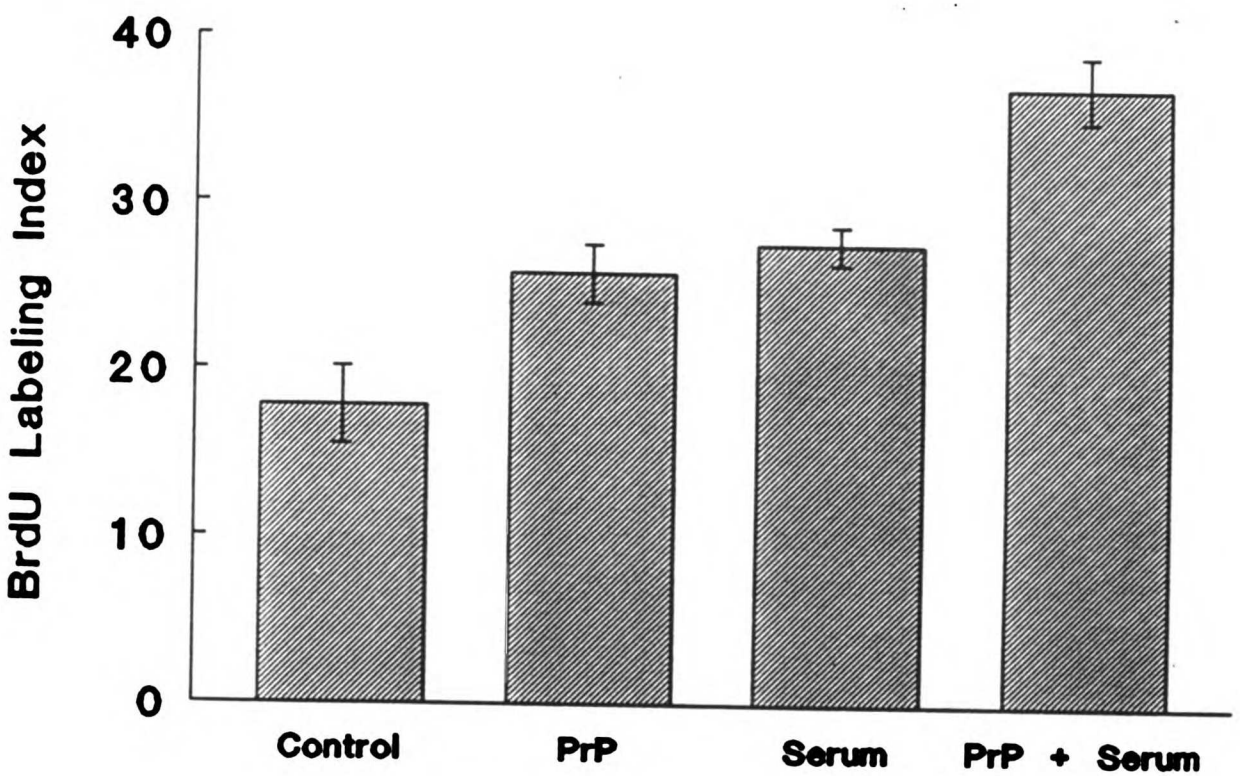

Figure #6: BrdU labeling index in neonatal astrocytes exposed to exposed to either medium without fetal calf serum (FCS) - *control*, medium plus 0.1 µg/ml PrP 27-30 - *PrP*, medium plus FCS - *Serum*, or medium plus FCS and 0.1 µg/ml PrP 27-30 - *PrP + Serum* - for 24 hours.

References

1. Kellings, K., N. Meyer, C. Mirenda, *et al.*, *Further analysis of nucleic acids in purified scrapie prion preparations by improved return refocusing gel electrophoresis (RRGE).* J. Gen. Virol., 1992. **73**: p. 1025-9.

2. Meyer, N., V. Rosenbaum, B. Schmidt, *et al.*, *Search for a putative scrapie genome in purified prion fractions reveals a paucity of nucleic acids.* J. Gen Virol., 1991. **72**(37-49).

3. Prusiner, S.B., *Novel proteinaceous infectious particles cause scrapie.* Science, 1982. **216**: p. 136-44.

4. Oesch, B., D. Westaway, M. Wälchli, *et al.*, *A cellular gene encodes scrapie PrP 27-30 protein.* Cell, 1985. **40**(735-46).

5. Borchelt, D.R., M. Scott, A. Taraboulos, *et al.*, *Scrapie and cellular prion protein differ in their kinetics of synthesis and topology in cultured cells.* J. Cell. Biol., 1990. **110**: p. 743-52.

6. McKinley, M.P., A. Taraboulos, L. Kenaga, *et al.*, *Ultrastructural localization of scrapie prion protein in cytoplasmic vesicles of infected culture cells.* Lab. Invest., 1991. **65**: p. 622-30.

7. Meyer, R.K., M.P. McKinley, K.A. Bowman, *et al.*, *Separation and properties of cellular and scrapie prion proteins.* Proc. Natl. Acad. Sci., 1986. **83**: p. 2310-4.

8. Gabizon, R., M.P. McKinley, D.F. Groth, *et al.*, *Immunoaffinity purification and neutralization of scrapie prion infectivity.* P.N.A.S., 1988. **85**(18): p. 6617-21.

9. Diringer, H., H. Gelderblom, H. Hilmert, *et al.*, *Scrapie infectivity, fibrils and low molecular weight protein.* Nature, 1983. **306**: p. 476-8.

10. Bolton, D.C., M.P. McKinley, and S.B. Prusiner, *Identification of a protein that purifies with the scrapie prion.* Science, 1982. **218**: p. 1309-11.

11. Stahl, N., M.A. Baldwin, R. Hecker, *et al.*, *Glycosylinositol phospholipid anchors of the scrapie and cellular prion protien contain sialic acid.* biochem., 1992. **31**: p. 5043-53.

12. Lopez, C.D., C.S. Yost, S.B. Prusiner, *et al.*, *Unusual topogenic sequence directs prion protein biogenesis.* Science, 1990. **248**(4952): p. 226-9.

13. Borchelt, D.R., A. Taraboulos, and S.B. Prusiner, *Evidence for synthesis of scrapie prion protein in the endocytic pathway.* J. Biol. Chem., 1992. **267**: p. 6188-99.

14. Taraboulos, A., A.J. Raeber, D.R. Borchelt, *et al.*, *Synthesis and trafficing of prion proteins in cultured cells.* Mol. Biol. Cell., 1992. **3**: p. 851-63.

15. Caughey, B. and R. G.J., *The scrapie-associated form of PrP is made from a cell surface precursor that is both protease- and phospholipase sensitive.* J.Biol. Chem., 1991. **266**: p. 18217-23.

16. DeArmond, S.J., M.P. McKinley, R.A. Barry, *et al.*, *Identification of prion amyloid filaments in scrapie-infected brain.* Cell, 1985. **41**: p. 221-35.

17. DeArmond, S.J., W.C. Mobley, D.L. DeMott, *et al.*, *Changes in the localization of brain prion proteins during scrapie infection.* Neurology, 1987. **37**: p. 1271-80.

18. Jendroska, K., F.P. Heinzel, M. Torchia, *et al.*, *Proteinase-resistant prion protein accumulation in Syrian hamster brain correlates with regional pathology and scrapie infectivity.* Neurology, 1991. **41**: p. 681-4.

19. Taraboulos, A., K. Jendroska, D. Serban, *et al.*, *Regional mapping of prion protein in brain.* P.N.A.S., 1992. **89**: p. 7620-4.

20. Hecker, R., A. Taraboulos, M. Scott, *et al.*, *Replication of distinct prion isolates is region specific in brains of transgenic mice and hamsters.* Genes & Development, 1992. **6**: p. 1213-28.

21. DeArmond, S.J., K. Kristensson, and R.P. Bowler, *PrPSc causes nerve cell death and stimulates astrocyte proliferation: a paradox.* Progress in Brain Research, 1992. **94**: p. 437-46.

22. Bolton, D.C., R.K. Meyer, and S.B. Prusiner, *Scrapie PrP 27-30 is a sialoglycoprotein.* J. Virol., 1985. **59**: p. 596-606.

23. Bruce, M.E., A.G. Dicinson, and H. Fraser, *Cerebral amyloidosis in scrapie in the mouse: effect of agent strain and mouse genotype.* Neuropathol. Appl. Neurobiol., 1976. **2**: p. 471-8.

24. Fraser, H. and M. Bruce, *Argyrophilic plaques in mice inoculated with scrapie from particular sources.* Lancet, 1973. : p. 617-8.

25. Prusiner, S.B., M. Scott, D. Foster, *et al.*, *Transgenic studies implicate interactions between homologous PrP isoforms in scrapie prion replication.* Cell, 1990. **63**: p. 673-86.

26. Scott, M., D. Foster, C. Mirenda, *et al.*, *Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques.* Cell, 1989. **59**: p. 847-57.

27. DeArmond, S.J., S.-L. Yang, A. Lee, *et al.*, *Three distinct scrapied prion isolates exhibit different patterns of PrPSc accumulation.* P.N.A.S., in press. .

28. Oesch, B., D.B. Teplow, N. Stahl, *et al.*, *Identification of cellular proteins binding to the scrapie prion protein.* Biochemistry, 1990. **29**(24): p. 5848-55.

29. Stahl, N., D.R. Borchelt, K. Hsiao, *et al.*, *Scrapie prion protein contains a phosphatidylinositol glycolipid.* Cell, 1987. **51**: p. 229-40.

30. Prusiner, S.B., M.P. McKinley, K.A. Bowman, *et al.*, *Scrapie prions aggregate to form amyloid-like birefringent rods.* Cell, 1983. **35**: p. 349-58.

31. Dichter, M.A., *Rat cortical neurons in cell culture: culture methods, cell morphology, electrophysiology, and synapse formation.* Brain Res., 1978. **149**: p. 279-93.

32. Eng, L.F., E. Stocklin, and Y.L. Lee, *Astrocyte culture on nitrocellulose membranes and plastic: detection of cytoskeletal proteins and mRNA's by immunocytochemistry and in situ hydridization.* J. Neurosci. Res., 1986. **16**: p. 219-50.

33. McKinley, M.P., S.J. DeArmond, M. Torchia, *et al.*, *Acceleration of scrapie in neonatal Syrian hamsters.* Neurology, 1989. **39**(10): p. 1319-24.

34. Martinou, J.C., D.L. Falls, D.D. Fischback, *et al.*, *Acetylcholine receptor-inducing activity stimulates expression of the epsilon-subunit gene of the muscle acetylcholine receptor.* P.N.A.S., 1991. **88**(17): p. 7669-73.

35. Gabriel, J.M., B. Oesch, H. Kretzschmar, *et al.*, *Molecular cloning of a candidate chicken prion protein.* P.N.A.S., 1992. **89**(19): p. 9097-101.

36. Laduron, P.M., *Criteria for receptor sites in binding studies.* Biochemical Pharmacology, 1984. **33**(6): p. 833-9.

37. Masters, C.L. and E.P. Richardson, *Subacute spongiform encephalopthy (Creutzfeldt-Jakob disease): the nature and progression of spongiform change.* Brain, 1978. **101**: p. 3333-44.