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# Differences in structure and function between human and murine tau☆

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ABSTRACT

The main difference between the primary structures of human and mouse tau can be found at the N-terminal end of the protein. Residues 17 to 28 in human tau are not present in the mouse form of the molecule. Here we tested the capacity of these human tau residues to bind to specific proteins. Several proteins were observed to bind to these residues. Among those that showed the greatest binding were three related to energetic processes: enolase, glyceraldehyde 3 phosphate dehydrogenase and creatine kinase B. The latter did not bind to tau from brain extracts taken from patients with Alzheimer's disease (AD). This lack of binding could be due to the modification of CKB by oxidation in AD.

## 1. Introduction

Tau is a microtubule-associated protein found mainly in the neurons of vertebrates (for a review see [1]). Tau of distinct origin shows some variability in primary sequence. By splitting the tau molecule into two halves, greater variability is observed in the N-terminal than in the C-terminal half [2].

Many studies of tau have been performed using the mouse or human form, despite differences between the primary sequences of these two proteins (Fig. 1). These differences are present mainly in the N-terminal halves [3,4]. Indeed, human tau is differentiated from the mouse form by the presence of an additional peptide containing amino acids from residue 17–28, inclusive.

Since the major difference between human and mouse tau is the presence of these residues (17–28) in the former, we tested whether this specific domain results in functional differences between the two forms of tau.

Here we sought to identify proteins with the capacity to interact with this human tau-specific domain. We found that three proteins, namely creatine kinase B (CKB), gamma enolase and glycerol-3 phosphate dehydrogenase, bound to the human peptide. One of them, CKB, did not bind when in an oxidized form. Of note, the oxidized form of CKB is present in the brains of patients with AD but not in those of controls [5,6].

## 2. Material and methods

### 2.1. Materials

Synthetic human tau peptide containing residues 16 to 26 was obtained from Abyntek, Spain. Mouse tau was isolated as previously described [7]. The human tau isoform (tau 4 + 2) was isolated as indicated [8]. CKB and polyclonal rabbit antibody were obtained from Abcam (Spain). Brain extracts were obtained from autopsies on non-demented controls and AD patients performed at the Hospital of Bellvitge (Barcelona, Spain), following national laws and international ethical and technical guidelines on the use of human samples for biomedical research purposes. Information on the subjects involved in this study is included in Table 1.

### 2.2. Methods

#### 2.2.1. Affinity chromatography

Human tau peptide (residues 16 to 26), full length human tau or

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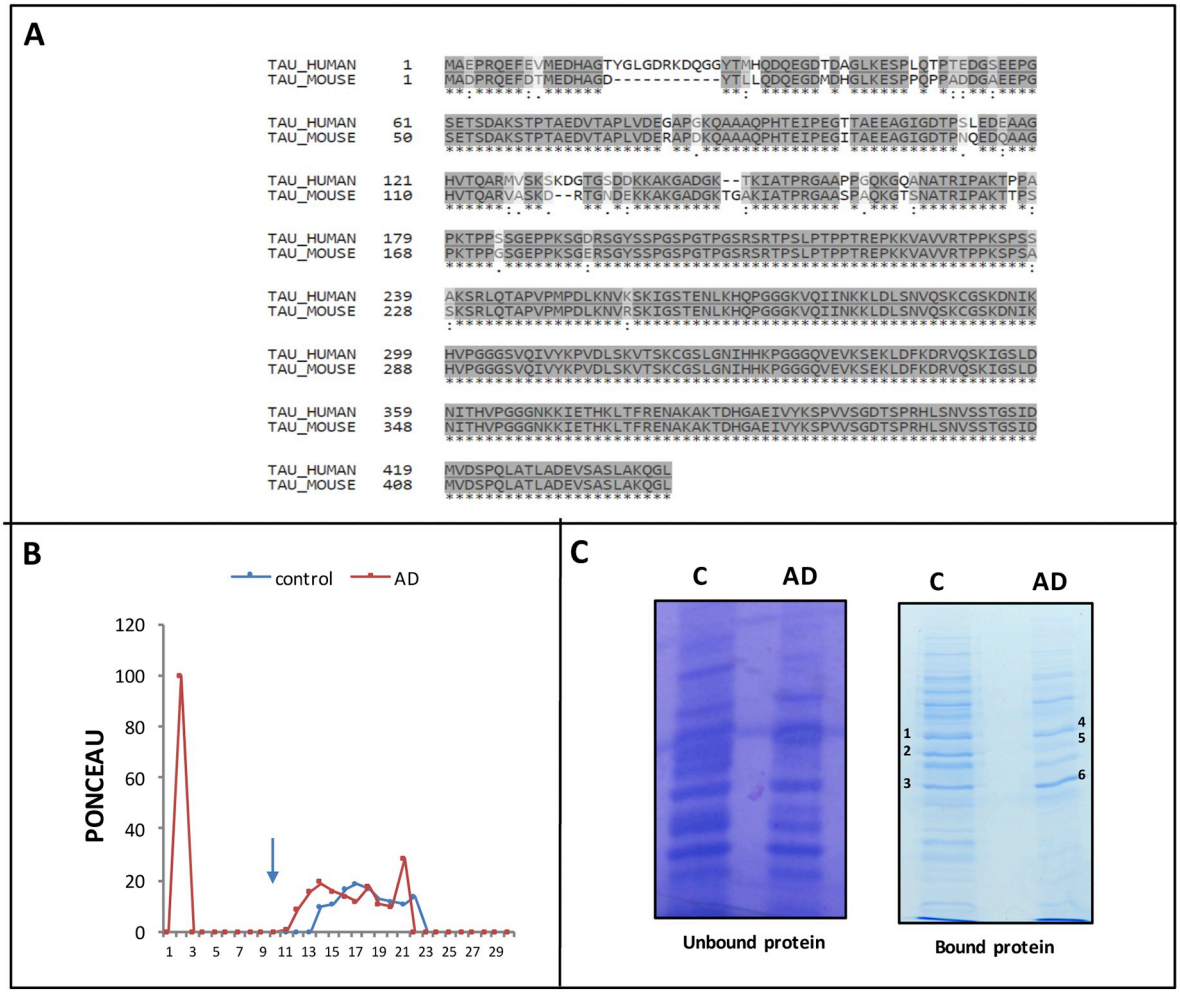


**Fig. 1.** Proteins bound to human tau peptide 18–28.
A) Differences between mouse and human tau proteins. Sequence alignment of human Tau (entry UniProt number P10636-8) and mouse tau (entry UniProt number sequence P10637-2) was done using the Clustal Omega program from the UniProt website. In the figure, the same amino acids are highlighted in dark gray (*), while conservative amino acids are highlighted in light gray (:) or less conservative (.).
B) Synthetic tau residues 18–28 was coupled to CNBr-activated Sepharose 4B, and brain extracts from control subjects or patients with Alzheimer's disease were loaded onto the column and washed with buffer A (see Methods section). Bound protein was eluted by addition of O.1 M NaCl in buffer A (marked with an arrow). The amount of total protein in each fraction was measured by Ponceau red followed by densitometry. The relative values are indicated in the ordinate.
C) Characterization of the proteins bound to human tau peptide. Gel electrophoresis was performed to characterize the unbound and bound proteins from brains extracts of controls subjects (C) and patients with Alzheimer's disease (AD). Numbers 1 to 6 indicate the most abundant proteins bound to the column.

**Table 1**
Table shows information on human brain tissue samples used.

| | Code | Age | Gender | Post-mortem interval | Braak-Tau | Area |
|---|---|---|---|---|---|---|
| AD | 1 | 93 | Male | 3 h | V | FrontalCx |
| | 2 | 81 | Female | 5 h 15 min | V | FrontalCx |
| | 3 | 85 | Male | 12 h 10 min | VI | FrontalCx |
| | 7 | 86 | Male | 04 h 15 min | V | FrontalCx |
| | 8 | 75 | Female | 11 h 30 min | V | FrontalCx |
| | 9 | 87 | Female | 5 h 15 min | V–VI | FrontalCx |
| CON | 4 | 41 | Male | 11 h 35 min | 0 | FrontalCx |
| | 5 | 51 | Female | 4 h | 0 | FrontalCx |
| | 6 | 54 | Female | 8 h | 0 | FrontalCx |
| | 10 | 47 | Male | 04 h 55 min | 0 | FrontalCx |
| | 11 | 75 | Male | 03 h 00 min | 0 | FrontalCx |
| | 12 | 52 | Female | 05 h 45 min | 0 | FrontalCx |

Information regarding code, age, gender, postmortem interval, Braak-Tau stage and cerebral area of each sample used is indicated.

mouse tau were covalently linked to a Sepharose 4B column [9]. Human brain extracts in buffer A (0.1 M MES, pH 6.4, 0.5 mm Mg $Cl_2$, 2 mm EGTA) were chromatographed on these columns in buffer A, and the protein retained was eluted by adding 0.5 M NaCl in buffer A [9].

### 2.2.2. *In-gel digestion*

After drying, gel bands or spots were destained in acetonitrile:water (ACN:H2O, 1:1). They were then reduced and alkylated (disulfide bonds from cysteinyl residues were reduced with 10 mM DTT for 1 h at 56 °C, and then thiol groups were alkylated with 50 mM iodoacetamide for 1 h at room temperature in darkness) and digested in situ with sequencing grade trypsin (Promega, Madison, WI), as described by Shevchenko et al. [10] with minor modifications [11]. The gel pieces were shrunk by removing all liquid using sufficient ACN. Acetonitrile was pipetted out, and the gel pieces were dried in a SpeedVac. The dried pieces were re-swollen in 50 mM ammonium bicarbonate pH 8.8 with 12.5 ng/μl trypsin for 1 h in an ice-bath. The digestion buffer was removed, and gels were covered again with 50 mM $NH_4HCO_3$ and incubated at 37 °C for 12 h. Digestion was stopped by the addition of 1% TFA. Whole supernatants were dried and then desalted onto ZipTip C18

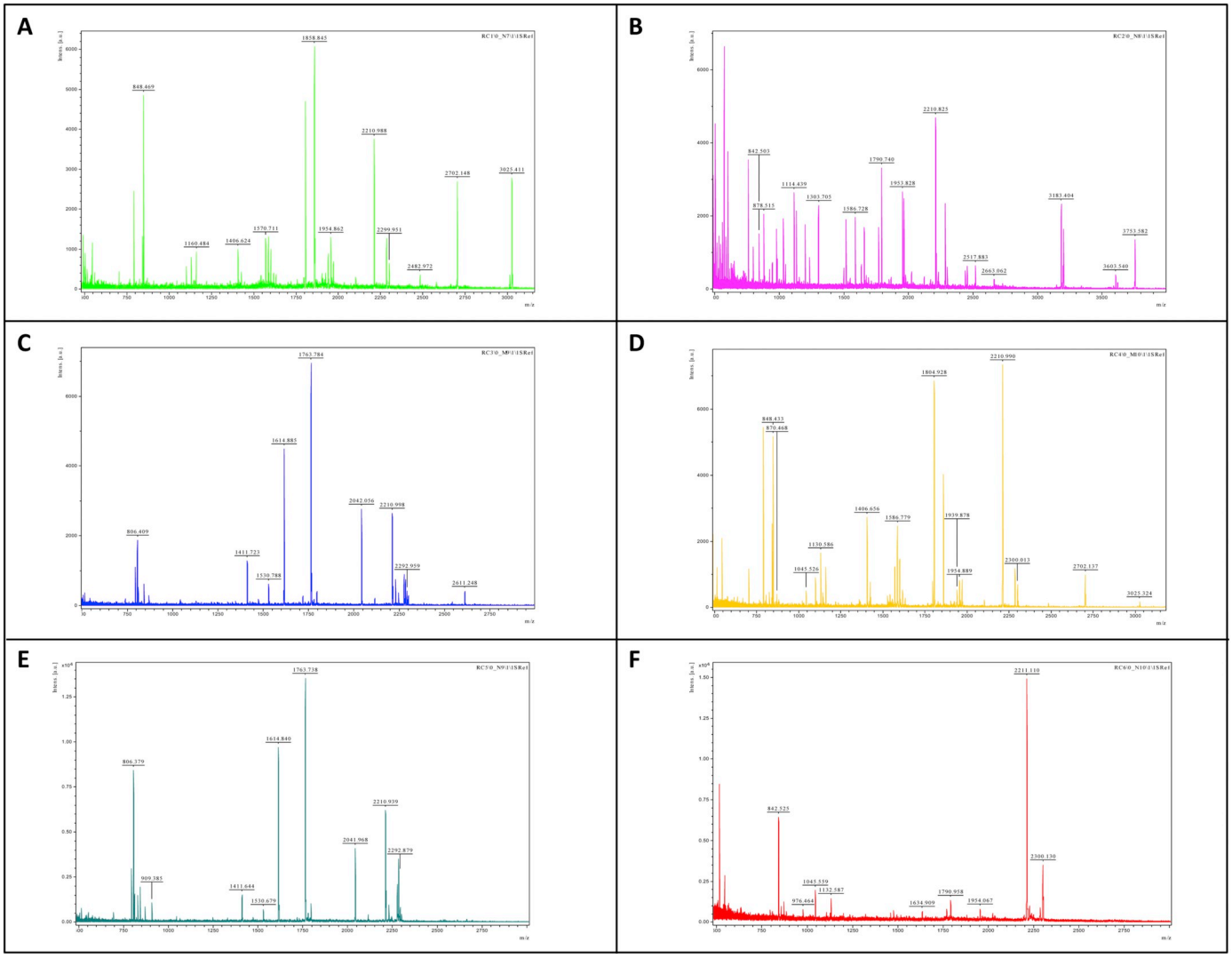


**Fig. 2.** Identification of proteins bound to human tau peptide.
The proteins numbered 1 to 6, from Fig. 1, were analyzed by mass spectrometry and the spectra obtained are shown.
A) Protein 1 (gamma-enolase)
B) Protein 2 (creatine kinase B and actin)
C) Protein 3 (glyceraldehyde 3 phosphate dehydrogenase)
D) Protein 4 (gamma-enolase)
E) Protein 6 (glyceraldehyde 3 phosphate dehydrogenase)
F) Protein 5 (actin).

Pipette tips (Millipore) followed by the mass spectrometric analysis.

*2.2.3. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis*

Peptide mass fingerprinting was conducted as previously described [12] using an Autoflex™ (Bruker Daltonics, Bremen, Germany) mass spectrometer in positive ion reflector mode and using 2,5-dihydroxybenzoic acid as matrix and an AnchorChip™surface target (Bruker Daltonics). Peak identification and monoisotopic peptide mass assignation were performed automatically using Flexanalysis™software, version 2.2 (Bruker Daltonics). Database searches were performed using MASCOT http://matrixscience.com [13] against the NCBI non-redundant protein sequence database http://www.ncbi.nih.gov. The following search parameters were used: tolerance of two missed cleavages, carbamidomethylation (Cys) and oxidation (Met) as fixed and variable modifications, respectively, and peptide tolerance at 100 ppm after close-external calibration. A significant MASCOT probability score ($p < 0.05$) was considered a condition for successful protein identification.

*2.2.4. Protein oxidation detection*

The OxyBlot Protein Oxidation Detection kit (S7150, Millipore) was used to detect oxidized proteins, as indicated in [14].

*2.2.5. Immunoprecipitation*

The immunoprecipitation protocol was carried out following commercial instructions (Immunoprecipitation Kit; Protein G; ROCHE, Cat No. 11 719 386 001) for solid tissue samples. Tissue was washed with PBS and then with lysis buffer (50 mM Tris-HCI, pH 7.5; 150 mM NaCI; 1% Nonidet P40; 0.5% sodium deoxycholate; supplemented with protease inhibitors) to achieve a concentration of 12 mg protein/ml. A preclearing step was then introduced to reduce background caused by non-specific adsorption. The extracts were used for co-immunoprecipitation by incubation with a mix of tau antibodies (Tau46-1/100- and 7.51-1/100-). After washing, the proteins were eluted from the beads by incubation with 50 µl SDS loading buffer, and 20-µl

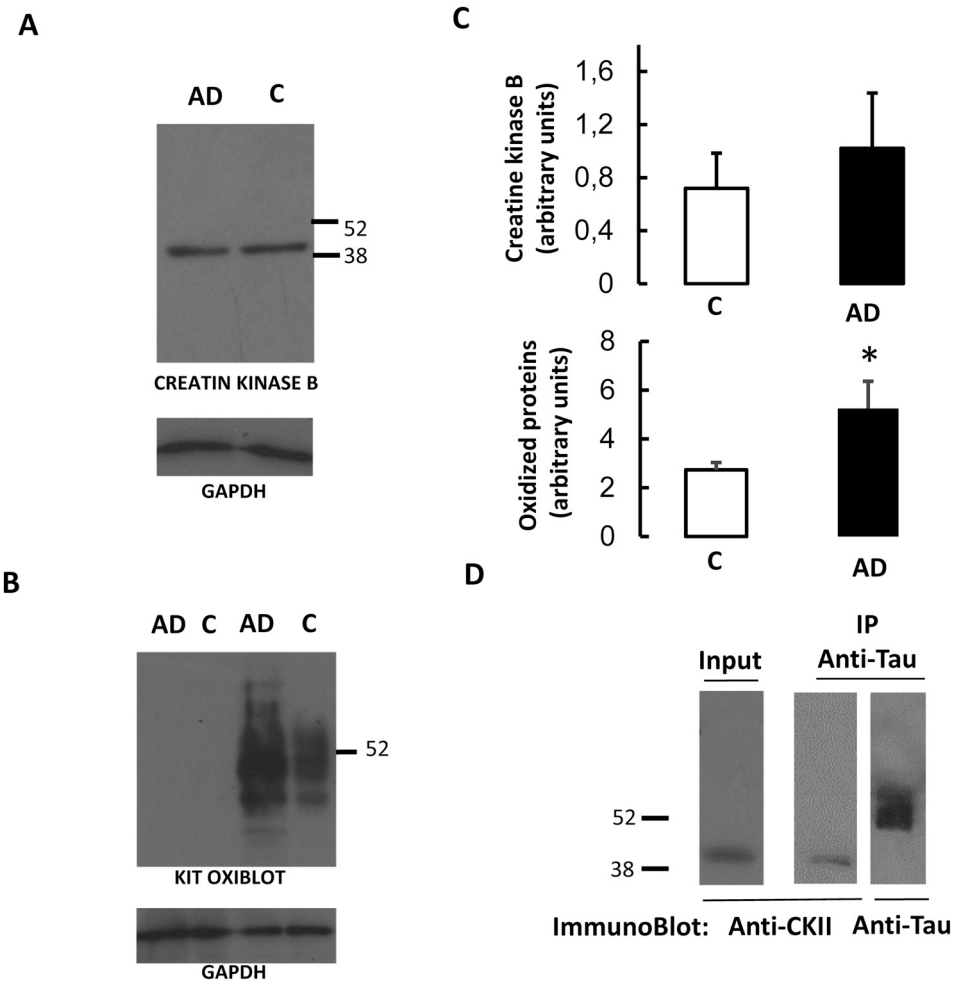


**Fig. 3.** Oxidized proteins in human brain cell extracts.
A) Gel electrophoresis showing the mobility of creatine kinase B identified by using a specific antibody against the protein in human brain extracts from control subjects (C) and patients with Alzheimer's disease (AD).
B) Oxyblot showing oxidized proteins from human brain extracts from C and patients with AD. The left side shows one aliquot that has not been subjected to the derivatization reaction (see Methods section). The amount of GAPDH in the fractions was determined using a specific antibody against the protein and used as a loading control.
C) Quantification of CK shown in A (n = 6) and oxidized proteins shown in B (n = 3) Student's *t*-test, *p < 0.05). Histograms show arbitrary unit of DO of CKB or oxidized proteins/GADPH in control and AD samples.
D) Human control sample was immunoprecipitated with anti-tau antibodies Tau43 and 7.51. Homogenates before immunoprecipitation (Input) and immunoprecipitates (IP) were analyzed by SDS-PAGE and immunoblotting (IB) using the indicated antibodies.

samples were analyzed by SDS-PAGE. Proteins were detected by incubation of individual membranes for 2 h with the respective antibody diluted in 5% non-fat milk.

*2.2.6. Dot blot analysis*

The presence of CKB in the fractions eluted with salt in the affinity chromatography analysis was determined by placing 5 µl of each fraction on nitrocellulose paper, followed by incubation with a specific antibody (Abcam) against CKB.

*2.2.7. Statistical analysis*

The data (n = 3–6) are presented as mean values ± S.E. Statistical analyses of data were performed by applying a Student's *t*-test for each statistical comparison. Significance was set at $p \leq 0.05$.

## 3. Results

### *3.1. Human proteins that bind to human tau peptide containing residues 16–26*

To test for proteins that specifically bind to human tau residues 16–26, we used brain extracts from non-demented control subjects (C) and from patients with Alzheimer's disease (AD). These extracts were chromatographed onto a column containing tau peptide 16–26 linked to Sepharose. A large proportion of the proteins present in the C and AD brain extracts did not bind to the column (Fig. 1B) and showed an electrophoretic pattern similar to that of unfractionated brain extracts (Fig. 1C). After exhaustive washing of the column, bound proteins (from C and AD samples) were eluted with an increased salt concentration. These proteins were characterized by gel electrophoresis. Six bands (see Fig. 1C) were analyzed and some proteins present in them were identified by mass spectrometry. Bands 1 and 4 were identified as gamma-enolase, band 2 as creatine kinase B (CKB) and actin, bands 3 and 6 as glycerol-3 phosphate dehydrogenase (G3PDH), and band 5 as actin (Fig. 2).

The main difference between the bound proteins in C and AD extracts was the presence of CKB in the former but not in the latter (Fig. 2). However, when we measured the amount of CKB in the two extracts, using an antibody against this protein, no significant differences were found (Fig. 3A). The differences in the affinity chromatography analysis could be explained by a modification of CKB in AD but not in C samples. Indeed, CKB is oxidized in the brains of AD patients [5,6]. A protein oxidation analyses using oxyblot detection kit (Fig. 3B) revealed the presence of a protein, mainly in AD samples, containing carbonyl groups introduced into protein by oxidative reactions and with the same electrophoretic mobility as CKB. This finding suggests that CKB in its oxidized form is unable to bind to tau peptide residues 16–26. We then immunoprecipitated human tau from control samples. CKB was successfully co-precipitated with human tau (Fig. 3C) demonstrating that CKB interacts with human tau.

### *3.2. Can mouse tau bind to CKB?*

Since murine tau lacks the human-specific residues 17–28, we tested the capacity of mouse tau to bind to CKB. To this end, we used affinity chromatography, in which mouse tau was covalently linked to the resin. In parallel, as a positive control, affinity chromatography with human tau was performed. In both cases, the procedure indicated in Fig. 1 was

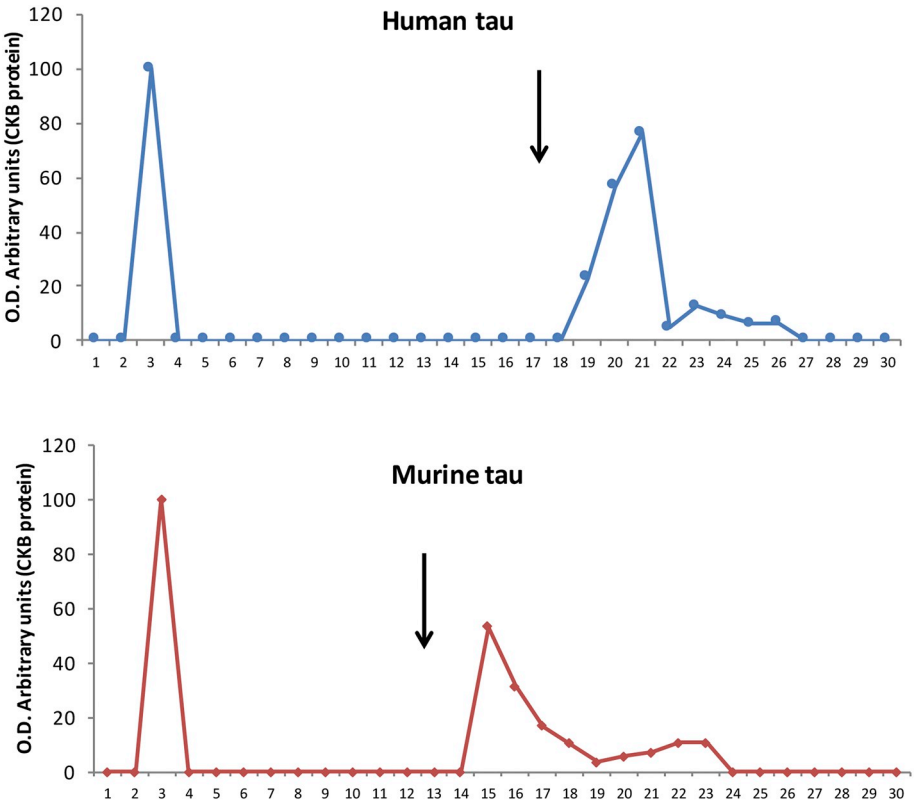


**Fig. 4.** Binding CKB to human or murine tau.
Affinity chromatography like that shown in Fig. 1B was performed but in this case A) whole human tau or B) whole murine tau was covalently linked to the column. A cell extract from human brain was loaded onto the column and CKB protein was determined by means of a dot blot and using a specific antibody, followed by densitometry of the dots.

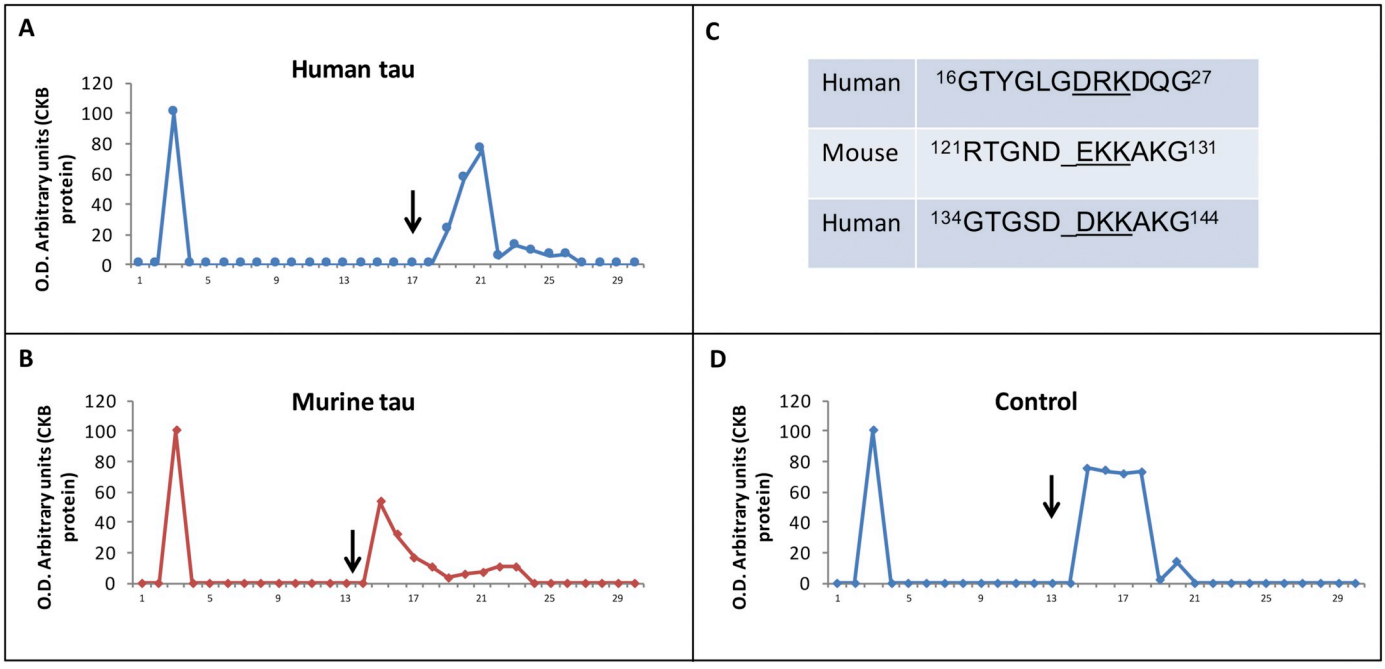


**Fig. 5.** Binding of CKB to murine residues 121–131.
A) Sequence comparison of human peptide residues 16–26 with mouse peptide residues 121–131 or human peptide residues 134–144.
B) Affinity chromatography like that shown in Fig. 1B. In this case tau murine residues 121–131 were linked to the column, and the CKB protein bound to the column was determined as in Fig. 4.

followed, and the elution of the bound CKB was analyzed by dot blot to test its interaction with both tau forms.

Mouse tau bound to CKB but with a lower affinity than human tau since it eluted at a lower salt concentration (Fig. 4). This result supports the involvement of human tau peptide in the binding of CKB; however, it also suggests that human (and mouse) tau holds a weaker additional binding site for CKB.

To explain binding of CKB to mouse tau we postulated that an additional tau domain was involved. Thus, we observed that residues 121–131 in mouse and 134–144 in human tau (Fig. 5A) as human tau residues 16–26 have basic residues that may bind to any of several acidic motifs, EDE, DEE, EED, DD or DE present in the primary structure

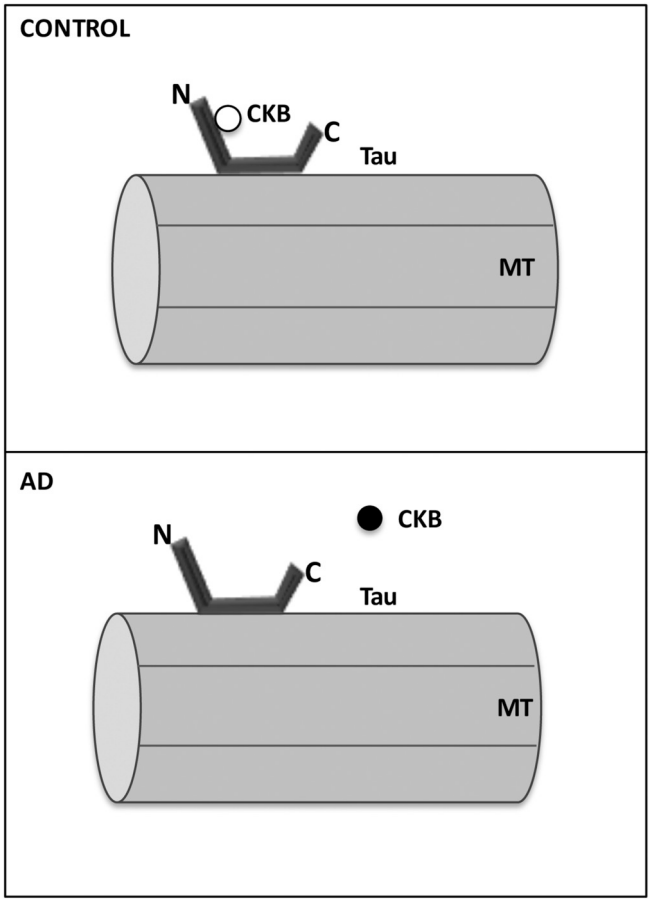


**Fig. 6.** Model for CK-B binding to microtubule-associated tau protein.
A) CKB (○) protein can bind to tau and be transported by microtubules (MT). B) The binding to MT does not take place in AD, probably due to the modification (●) of CKB caused by oxidation.

of CKB. We then hypothesized that this mouse tau sequence could be involved in the binding of tau to CKB. Fig. 5B shows an affinity chromatography using mouse tau peptide (residues 121–131) revealing an interaction with CKB.

## 4. Discussion

Here we have examined the potential functions involving human tau peptide residues 17 to 28. A search of various data banks revealed that this sequence was not present in other proteins, and we were only able to find a motif (DXXD) that is involved in caspase cleavage [15,16]. Human tau truncation at residue 26 has been reported, and this residue is part of a DXXD motif [17]. We then tested whether the human tau peptide (residues 16–26) participates in the interaction of human tau with other brain proteins (Fig. 6).

We found that three proteins: CKB, gamma-enolase and glyceraldehyde 3-phosphate dehydrogenase, bound to the human tau peptide comprising residues 16 to 26. CK-B is a brain protein that phosphorylates creatine (carbamimidoyl (methyl) amino acetic acid) in the presence of ATP [18]. Enolase is a critical enzyme in the glycolytic pathway. It has three different subunits, α, β and γ, and acts as a dimer. It is located mainly in the cytoplasm, and the dimer γγ is found mostly in mature neurons [19]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is an oxidoreductase that catalyzes the conversion of glyceraldehyde 3 phosphate to D-glycerate 1,3 bisphosphate in one of the steps of the glycolytic pathway.

These three proteins are related to energetic processes involving the production of ATP or NADH. These processes could be associated with neuronal functions like axonal transport, which seems to be impaired in neurodegenerative disorders like AD, a disease that correlates with a progressive energy deficiency in the central nervous system [20]. Additionally, due to its high energy demands, the brain is highly susceptible to oxidative imbalance and changes in the level of ATP may correlate with neurodegeneration [21]. We have focused our study mainly in CKB because the main difference between the bound proteins in control and AD extracts to human N-terminal peptide was the presence of CKB in control but not in AD samples.

Human tau (16-26)-binding proteins may play a role in axonal transport. In this regard, the CKB/phosphocreatine complex could facilitate a temporal energy buffer by producing the ATP required for axonal transport [22]. Thus creatine pretreatment protects cortical axons from energy depletion in vitro [23]. Also, both, enolase and CKB can move in axons, a phenomenon associated with the component b of axonal transport [24]. Furthermore, GAPDH has been implicated in rapid axonal transport [25].

Our data can also explain the difficulty in reproducing phenomena associated with AD in mouse models. Indeed, our findings demonstrate a lower affinity of CKB for mouse tau than for human tau. These data, together with the lower affinity of the CKB observed in human samples with AD, may explain some of the phenomena associated with the disease. Furthermore, the presence of tau peptide (residues 17–28) in human could regulate the intramolecular interaction between N and C termini of the protein [26], which are modulated by microtubule interactions in living cells [27]. This interaction may compete with proteins like CKB, which bind to the tau peptide presents at the N-terminus.

Our study adds new proteins able to bind N-terminal human tau end. Thus, it has been recently published that deletion of amino acids studied here from longest human tau isoform alters binding of proteins as synapsin-1, synaptotagmin-1, some 14-3-3 proteins and Annexin A5, having the human form more affinity than the deleted form [28].

Proteins here described able to bind N-terminal end of human tau (residues 16–26) have been described to be involved in AD. AD may be caused by a progressive energy deficiency syndrome in the central nervous system [20]. In AD, CKB [5,6], enolase [29] and GAPDH [30] are modified by oxidation. In the case of GAPDH (a protein that interacts with APP), its enzymatic activity shows a significant decrease in this disease, as a result of oxidative modification [30] resulting in higher susceptibility of cells bearing oxidatively modified GAPDH.

## Transparency document

The Transparency document associated this article can be found, in online version.

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