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Taurine, an inducer for tau polymerization and a weak inhibitor for amyloid-β-peptide aggregation

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Abstract

Taurine is an abundant aminoacid present in brain. Its concentration is decreased in the brain of Alzheimer's disease (AD) patients. The chemical structure of taurine is similar to 3-amino-1-propanesulfonic acid, a known compound which interferes with beta-amyloid peptide aggregation. Here, we have tested if taurine show similar properties. Taurine slightly decreases beta-amyloid peptide aggregation at a milimolar concentration. At that concentration, taurine favours the assembly of tau protein into fibrillars polymers. Thus, it is proposed that the negative charge present in taurine may be involved in the binding to tau protein, facilitating its assembly. In addition, the possible role of taurine in Alzheimer disease is commented.

Keywords: Taurine; Tau polymerization; Alzheimer disease

Taurine, 2-aminoethane sulfonic acid, is one of the most abundant free aminoacids in the brain [10] where it may play a role as an osmoregulator, antioxidant, neuromodulator or may control calcium influx [12]. It has been also described that taurine may induce an increase in Cl^- conductance upon binding to $GABA_A$ receptors [7]. More recently, it has been indicated that taurine prevents the neurotoxicity of beta-amyloid peptide and that the neuroprotection is related to the activation of $GABA_A$ receptors [16].

Beta-amyloid peptide (Aβ) is the main component of the senile plaques, one of the aberrant structures, together with the neurofibrillary tangles, found in the brain of Alzheimer's disease (AD) patients. In AD a decrease in taurine concentration has been observed in the brain or in the cerebrospinal fluid of the AD patients [4,3]. On the other hand, neurofibrillary tangles are present inside taurine-expressing neurons, in AD patients [15].

Beta-amyloid peptide is toxic for neurons, in aggregated form [11]. Although, the type of aggregates showing a higher toxic effect remains under discussion [14], a proposed therapy for amyloid pathology in AD has been the search for inhibitors of amyloid aggregation or compounds that could interfere with those factors that facilitate amyloid aggregation. One of those factors that facilitates amyloid aggregation is heparin, a glycosaminoglycan. High molecular weight heparin, could associate to Aβ and facilitate the formation of amyloid fibrils [20], although heparin (or heparan sulfate) could also inhibit β-secretase activity [17]. On the other hand, it has been found that a low molecular weight form of heparin, enoxaparin, could reduce amyloid accumulation in a mouse model of AD [6]. In addition specific low molecular weight compounds, which mimic the anionic properties of glycosaminoglycans (GAG), could interfere with the binding of those GAGs with amyloid peptide. One of those compounds is Tramiprosate (3-amino-1-propane sulfonic acid), also known as 3-APS or Alzhemed™. This compound interferes with the aggregation of amyloid peptide, and it has been considered as a promising drug for the for the treatment of AD [8], being at the present a phase II study to target amyloid-beta with 3-APS in mild to moderate AD [1]. A large phase III for this compound has been already announced [2].

However, taurine is a compound structurally related to 3-APS. Indeed 3-APS is also named as homotaurine (Fig. 1), and although, it has been suggested that taurine has not antifibrillogenic activity [8], a direct experiment showing that, has not

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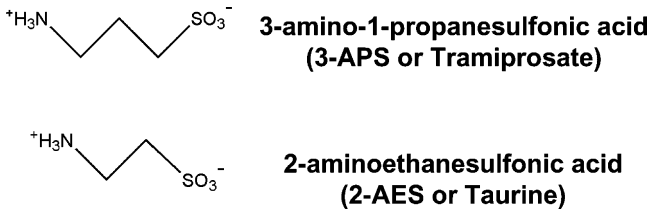

Fig. 1. Structure of 3-amino-1-propanesulfonic acid (3-APS or Tramiprosate), an 2-amino-ethanesulfonic acid (2-AES or taurine). The chemical structure of the compounds used in this study, 3-APS and 2-AES, is shown.

been yet carried out. In fact, taurine binds to beta-amyloid peptide in a similar way to that of 3-APS although, unlike 3-APS, taurine does not prevent the transition to β-sheet conformation of amyloid peptide [8].

To test if, indeed, taurine prevents or not the polymerization into fibrils of amyloid peptide, we have used amyloid peptide (0.1 mg/ml) comprising the residues 25–35, containing the region of beta-amyloid peptide with a higher capacity for self assembly; we have mixed it with taurine. Fig. 2 shows that in the absence of taurine (Fig. 2A), amyloid peptide aggregates, whereas in the presence of taurine (Fig. 2B) the aggregation decreases, as determined by electron microscopy. A higher decrease in amyloid aggregation was observed when the 3-APS (used as positive control [19]) was added (Fig. 2C). To quantify the amount of polymer in the absence or presence of taurine, measurement of turbidity (absorbance at 310 nm) of the aggregated protein was achieved. By taking as 100% the turbidity of aggregated amyloid peptide, in the absence of any added compound, we found a value for that turbidity of $66 \pm 21\%$ in the presence of taurine and a value of $38 \pm 7\%$, in the presence of 3-APS. Nevertheless, it could be argued that a turbidity measurement could not be a suitable method to determine the amount of aggregated beta-amyloid peptide. Thus, the polymerized fractions were centrifugated in an Airfuge at $100{,}000 \times g$ for 60 min. After centrifugation the amount of protein in non-polymerized form was determined by measuring the absorbance (at 230 and 260 nm) of the protein remaining in the

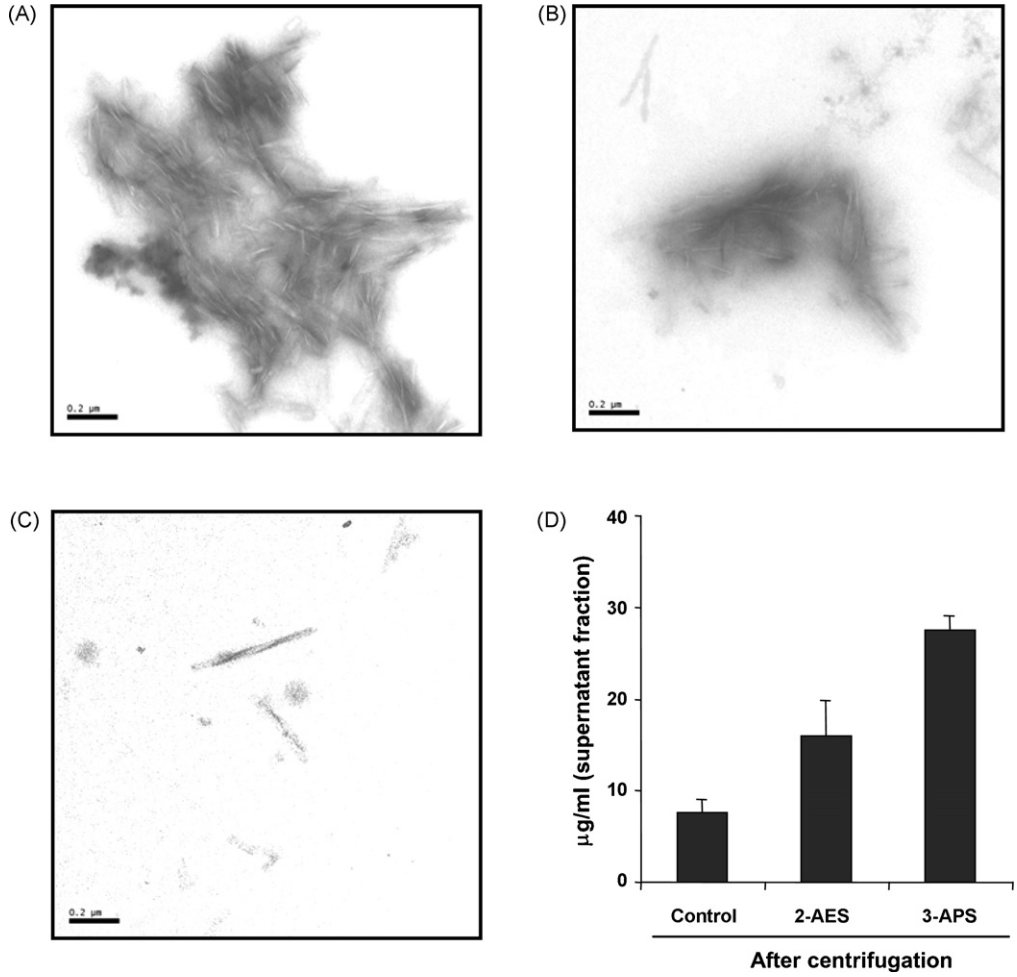

Fig. 2. Effect of taurine on amyloid peptide assembly. (A) Electronmicrograph of beta amyloid aggregates; (B) beta-amyloid aggregates incubated in the presence of 1 mM 2-AES; (C) beta-amyloid aggregates incubated in the presence of 1 mM 3-APS; (D) measurement of the protein remaining in unpolymerized form after incubation of beta amyloid peptide to aggregate. The absorbance at 230 nm of a solution of 0.1 mg/ml of beta amyloid peptide was measured (total protein). An absorbance of about 0.5, similar to that of other proteins at the same concentration, was obtained. This protein solution was incubated (48 h at 37 °C) to form aggregates in the absence (control) or the presence of 1 mM taurine (2-AES) or 3-APS. After the incubation, the mixtures were centrifuged in an Airfuge at $100{,}000 \times g$ for 60 min and the protein amount present in the supernatant was measured by absorbance at 230 and 260 nm, and the amount of protein was calculated by $\mu g/ml = 183A_{230} - 75.8A_{260}$, as indicated in Ref. [13].

supernatant fraction. We have previously observed a linear relationship between amount of protein (using serum albumin) and absorbance at 230 and 260 nm, by doing the following calculation: $\mu g/ml = 183A_{230} - 75.8A_{260}$ [13]. Fig. 2D shows that in the presence of taurine an increase, compared to the control, of amyloid peptide in unpolymerized form was found, being that increase higher in the presence of 3-APS (used as a positive control) [19].

It has been previously described [8] that 3-APS, but not taurine, prevents the transition to a beta sheet conformation of amyloid peptide. Thus, our results suggest that although taurine does not prevent that conformational change, it slightly interferes with amyloid aggregation.

Taurine is a natural occurring β-aminoacid, that can cross the blood brain barrier, and it is a normal component of the brain [12], although its concentration is decreased in AD patients [4]. Thus, it cannot be ruled out the use of taurine as a possible therapeutic agent to avoid amyloid peptide aggregation.

However, AD is characterized by the presence of two hallmarks; amyloid aggregates (previously commented), and tau aggregates, components of neurofibrillary tangles (NFTs). These NFTs are clusters of filamentous polymers of tau protein, in phosphorylated form [9]. Since GSK-3 has been described like one of the main tau kinases [5], the effect of taurine on tau modification by GSK-3 was tested in vitro. However, no differences in tau phosphorylation were observed in the presence or absence of taurine (data not shown). When, the effect of taurine on tau aggregation was studied (using recombinant tau protein corresponding to the longest human tau isoform [18]), it was found (Fig. 3), that taurine facilitates that aggregation, since fibrillar tau polymers were observed in the presence of taurine (Fig. 3B), whereas no filaments were observed in the absence of taurine (Fig. 3A). In previous experiments [19], it was described that 3-APS also acts like an inducer for tau assembly. In this way, 3-APS was used as a positive control for tau aggregation (Fig. 3C). A quantitation of the amount of tau polymers was done by looking at a sample of the protein remaining in the supernatant fraction after centrifugation to remove the assembled protein, by using the same criteria, determination of the absorbance at 230 and 260 nm, indicated for amyloid peptide. The analysis was done in the absence or in the presence of taurine (2-AES) or 3-APS. Tau protein, in the absence of any polymerization inducers, remains in unpolymerized form (unlike Aβ peptide). Thus, essentially all the protein remains in the supernatant fraction, whereas a

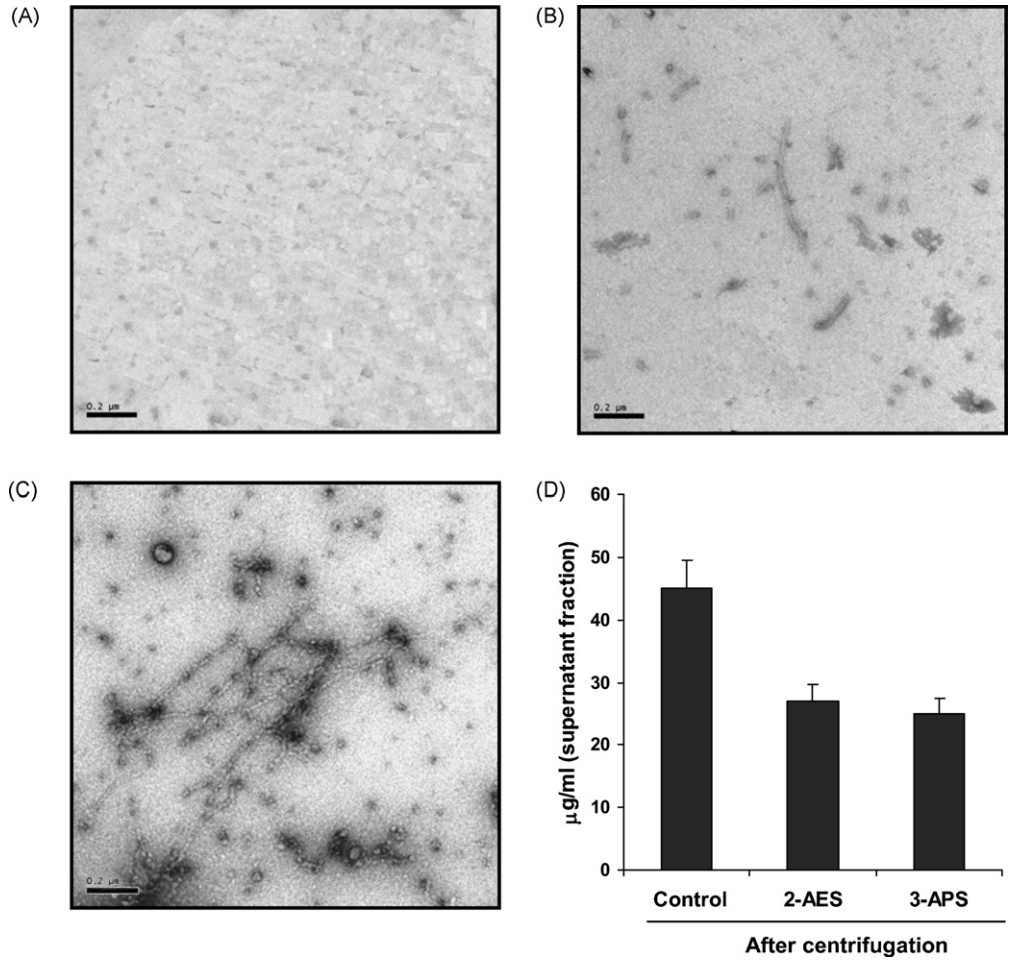

Fig. 3. Taurine as an inducer for tau polymerization. Tau protein was incubated, as indicated [18], in the absence (A), or the presence of 1 mM taurine (B) or 1 mM 3-APS (C). In addition, the amount of tau aggregates in the absence (control), or the presence of taurine (2-AES) or 3-APS, measured as indicated for amyloid peptide aggregation (by testing the amount of protein that remains in non-polymerized form after centrifugation) is shown (D).

decrease in that fraction was found in the presence of taurine (2-AES) or 3-APS. Fig. 3D shows that both, taurine (2-AES) and 3-APS, facilitates tau aggregation in a similar fashion.

Since, those molecules also induce the assembly of a tau protein fragment containing the basic region involved in microtubule binding (not shown), it can be suggested that the negative charge of taurine (or 3-APS) could bind to the residues of that tau peptide, inducing its assembly. In summary, taurine and 3-APS share some common characteristics like prevention of amyloid peptide aggregation (although at different extent), and the formation of tau polymers. In addition, since taurine prevents the neurotoxicity of beta amyloid peptide, this natural β-aminoacid could be a promising molecule for that pathological feature related to Alzheimer disease. However, its effect on tau aggregation deserves a further analysis.

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