Neurodegeneration induced by Aβ oligomeric fractions : Role of NMDA receptors and mGluR5.

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1 Introduction

Alzheimer disease (AD) affects mainly people over the age of 65 years, suffering from different clinical symptoms such as progressive decline in memory, thinking, language, and learning capacity. The toxic role of beta amyloid peptide (Aβ) has now shifted from insoluble Aβ fibrils to smaller, soluble oligomeric Aβ aggregates (AβO).

Role of NMDAR (Fig. 1)
NMDA receptors (NMDAR) are essential mediators of brain plasticity and are capable of converting specific patterns of neuronal activity into long-term changes in synapses structure and function that are thought to underlie higher cognitive functions. Excessive stimulation of NMDARs has been implicated in AD. Various mechanisms, from selective inhibition of extra-synaptic NMDARs to more effective inhibition of subunits have been proposed. Aβ has been shown to perturb synaptic functions by removing synaptic NMDARs.

Role of mGluR5 (Fig. 1)
Evidence for a direct mechanistic interaction between mGluR5 and Aβ was shown by Renner *et al.* (2010). Clustering of Aβ at the membrane greatly reduced the ability of mGluR5 to laterally diffuse from the synapse, resulting in facilitation of mGluR5-mediated signaling. Additionally, Ab binding to the neuronal surface of hippocampal cultured neurons from mGluR5 knockout mice was greatly reduced, suggesting that mGluR5 may play a reciprocal role in "scaffolding" Aβ oligomer cluster to the synapses.

Objectives
Using a well validated in vitro model of AβO injuries (on primary cortical neurons) (Callizot *et al.*, 2013), in this study, we deeply investigated the role of NMDAR subunits as well as mGluR5 in the neurotoxicity induced by AβO. Specific antagonists were used to assess the mode of action (MoA).

2 Methods

Culture of cortical neurons: Rat cortical neurons (E15) were cultured as described by Callizot *et al.*, 2013. The cells were seeded at a density of 30,000 per well in 96-well plates (for immuno-staining) pre-coated with poly-L-lysine and cultured at 37°C in an air (95%) – CO2 (5%) incubator. Six wells were used per condition.

Pharmacological treatments: The cortical neurons were treated with Aβ solution (10 µmol/L) after 11 days of culture. The human Aβ-1-42 preparation was done following the procedure (Callizot *et al.*, 2013). 1h before Aβ addition, the cells were incubated with the following inhibitors:

- Memantine: a low-affinity voltage-dependent uncompetitive antagonist at NMDAR, memantine is able to inhibit the prolonged influx of Ca2+ ions, particularly from extrasynaptic receptors.
- Ifenprodil: selective inhibitor of the NMDAR GluN2B subunit.
- MK-801 (dizocilpine): an uncompetitive antagonist of NMDAR.
- MPEP: 2-Methyl-6-(phenylethynyl)pyridine selective antagonist for the metabotropic glutamate receptor subtype mGluR5.

Immunostaining: After 24h of Aβ treatment, cells were fixed by a solution of ethanol (95%) acetic acid (5%). The cells were incubated with monoclonal anti-microtubule-associated-protein 2 (MAP-2). That was revealed with Alexa Fluor 488 goat anti-mouse IgG. The immuno-labelled cultures were examined with MetaXpress (Molecular Devices) at X 20 magnification. Survival of neuron was evaluated as well as neurite network reflecting the synaptic loss of neurons.

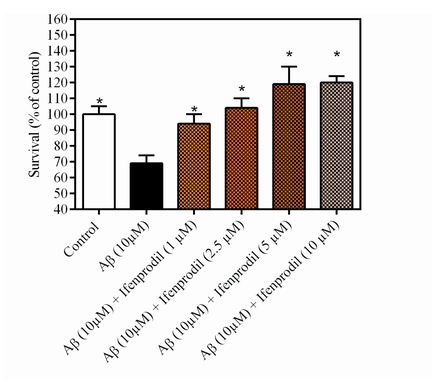
3 Results

Here we showed :

- NMDAR are highly involved in the AβO neurotoxicity as shown by full protective effect of MK801 (Fig. 4);
- NMDAR2B are highly involved in the AβO toxicity as shown by full protective effect of Ifenprodil (Fig. 2);
- Mainly NMDAR intrasynaptic receptors are involved as shown by the minor effect of Memantine (Fig. 3);
- mGluR5 play an important role in the AβO toxicity (Fig. 5).

Fig. 2

Effect of Ifenprodil (different concentrations) on cortical neuron survival after AβO application



Effect of Ifenprodil (different concentrations) on cortical neurite network after AβO application

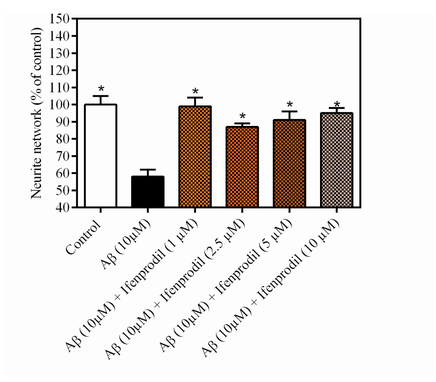
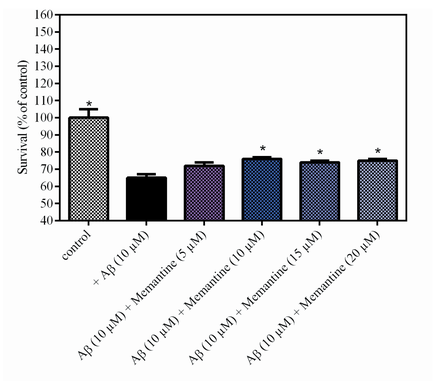


Fig. 3

Effect of Memantine (different concentrations) on cortical neuron survival after AβO application



Effect of Memantine (different concentrations) on cortical neurite network after AβO application

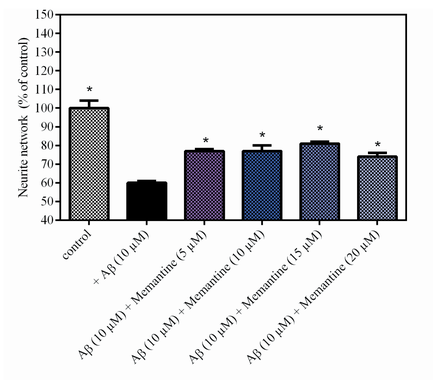
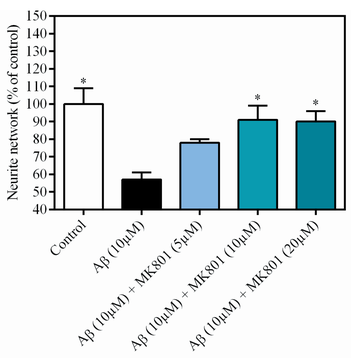


Fig. 4

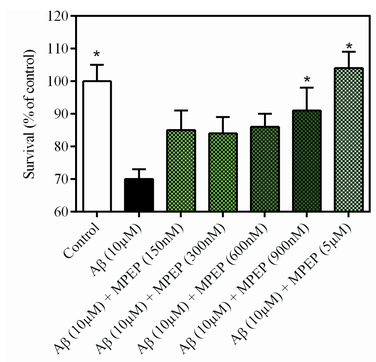
Effect of MK801 (different concentrations) on cortical neuron network after AβO application

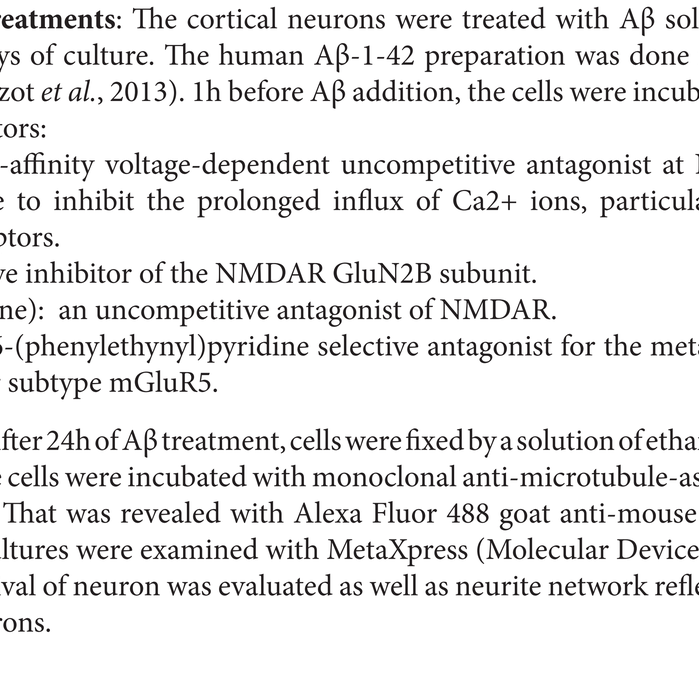


* $p < 0.05$ one way ANOVA followed by Dunnett's test

Fig. 5

Effect of MPEP (different concentrations) on cortical neuron survival after AβO application





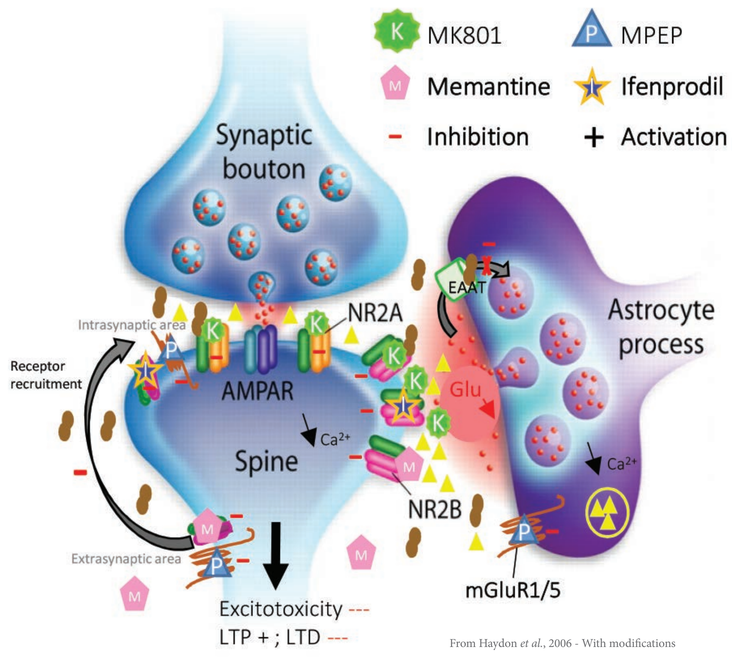
From Haydon *et al.*, 2006 - With modifications

Fig. 1: Hypothesis of AβO toxicity

4 Hypothesis

These results suggest that AβO:

1. Increases the glutamate concentration in the synaptic cleft by release it from presynaptic button and/or astrocytes or by inhibiting its reuptake (Fig. 6).
2. Induces a large NMDAR2B lateral diffusion.
3. Interacts with mGluR5, resulting in clusterisation of mGluR5 in the synaptic area and the over-activation of mGluR5 with subsequent pathological effects upon NMDAR function and Ca^{2+} homeostasis.
4. Induces a massive Ca^{2+} influx in the dendritic spine, increasing Long Term Depression process, excitotoxicity and finally neuronal death.



From Haydon *et al.*, 2006 - With modifications

Fig. 6: Protective effect of antagonists

Callizot *et al.*, (2013) J Neurosci Res. 2013;91(5):706-16 / Renner *et al.*, (2010) Neuron. 10;66(5):739-54