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Total impact factor: 36.846

*:= Publications used in the thesis.

Doctoral thesis summary

Effect of different sized β -amyloid oligomer aggregates on the neuronal excitability in the hippocampus, a complex, synergistic mechanism in the background of Alzheimer's disease, epilepsy and inflammatory processes

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INTRODUCTION

Neurodegenerative diseases form one of the largest problematic disease group world wide. The number of patients suffering of Alzheimer's disease is doubled every 5 years with the advance of the age, so the disease mostly involves elderly people. In regions with ageing populations (Europe, USA, Japan), the increase in number of patients with neurodegenerative diseases of old age is getting more and more pronounced, so it is becoming one of the most challenging problems for the healthcare systems.

Causal treatment of neurodegenerative diseases -mostly because of the lack of the knowledge of the pathomechanisms- is yet unsolved. At present only phenomenologic treatments are available, which involve slowing down the progress of the diseases, easing and delaying the appearance of the symptoms, so enhancing the quality of life of the patients, and prolonging their lives, but not providing a solution to cure these diseases. So a better understanding of the pathomechanisms and a disclosure of the earliest detectable disorders is essential. Basically two experimental approaches are given: examination of genetically modified β -amyloid ($A\beta$) overexpressing animals, or administration of $A\beta$ to healthy animals. One possibility that can get us closer to understanding the early effects of $A\beta$ among the experiments with administration of $A\beta$ is to disclose the acute effect of aggregates on neuronal function. Our work summarised in the present thesis is on one hand focusing on the problems of the application of different amyloid aggregates in experimental conditions, looking for early effects of the compound, on the other hand focusing on collecting data about new

Local administration of THIP (GABA_A δ subunit specific agonist), NO-711 (GAT-1 transporter specific antagonist) in the VB nuclei of the thalamus of Wistar rats via reverse-microdialysis.

Administration of antisense oligo-deoxy nucleotides (and missense controls) to decrease the expression of GABA_A δ subunits (gene silencing) via local microinjection in the VB of GAERS rats.

Examination of the effect of peripheral lipopolysaccharide administration on the thalamic and cortical proteome

Preparation of tissue samples, and 2D-DIGE experiments.

Production of preparative 2D gels after statistical analysis of the DIGE data.

Recovery of proteins from the spots that have shown statistically significant change, LC/MS identification of proteins.

Interpretive modelling of the proteomic data, *in silico* reconstruction of protein networks.

LIST OF PUBLICATIONS

1*: Orbán G, Völgyi K, Juhász G, Penke B, Kékesi KA, Kardos J, Czurkó A. Different electrophysiological actions of 24- and 72-hour aggregated amyloid-beta oligomers on hippocampal field population spike in both anesthetized and awake rats. *Brain Res.* 2010 Oct 1;1354:227-35. Epub 2010 Jul 24. PubMed PMID: 20659435. IF: 2.463

2*: Cope DW, Di Giovanni G, Fyson SJ, Orbán G, Errington AC, Lorincz ML, Gould TM, Carter DA, Crunelli V. Enhanced tonic GABA_A inhibition in typical absence epilepsy. *Nat Med.* 2009 Dec;15(12):1392-8. Epub 2009.

Redissolving of lyophilizates in artificial cerebro-spinal fluid, storage at -80 °C until later use.

Incubation at room temperature for 0, 24, 72 hours to let the solutions aggregate.

Post-checking of degree of aggregation in solutions used in the experiments

Atomic Force Microscopy imaging of the solutions.

Thioflavin-T fluorescence intensity measurements.

Examination of the effect of the solutions on hippocampal population spike genesis

Recording hippocampal population spikes *in vivo* in anesthetized and freely moving Spague-Dowley rats.

Administration of β -amyloid aggregate solutions during the experiments via intra-hippocampal microinjection.

Pharmacological study of the effect of enhanced tonic GABA_A inhibition in vivo

Fronto-parietal EEG recordings in freely moving Wistar and GAERS rats.

aspects of the Alzheimer's disease pathomechanism due to absence-like seizures and inflammation caused by the increasing amount of A β in the tissue.

One of the most important informations disclosed by our experiments was that acute experiments ran with amyloid aggregates revealed that instead of earlier claims the earliest effect of oligomers on excitability is not due to disturbances in synaptic transmission. Besides several recent data suggests that absence-like epilepsy, seizures can be observed in Alzheimer's diseased patients during the late progression of the disease. Above all inflammatory cytokines play a critical role in both the pathomechanism of Alzheimer's disease and the regulation of epileptoid activity.

Since the genesis of absence epileptic seizures can be ruled back to enhanced inhibition, and particular A β aggregates with a well defined degree of aggregation cause a decrease in excitability we also included a study on the pathomechanism of absence epilepsy, especially the cause of the change of the excitability of thalamo-cortical relay neurons. Later on we attempted to find common mechanisms of Alzheimer's disease, epilepsy and inflammatory reaction. This work can be claimed a pilot study first step, in which we attempt to reveal new information about Alzheimer's disease from multiple different approaches. Our work is justified by the fact that in the research field of Alzheimer's disease there has been a wide range of exploration nowadays, since traditional experimental approaches seem to have reached their maximum of possibilities. Considering these, our results generated more new questions than answers they gave, therefore

the classical coherence couldn't always be maintained. Despite of this, we always choose a critical and proposing way of discussion.

AIMS OF THE STUDY

To produce and use the most homogenous aggregate solutions possible, and to define the degrees of aggregation in the solutions used in the experiments as much as possible with the help of tools and results of recent biophysical studies

To compare the effect of solutions with different degrees of aggregation.

To establish a complementary model to disclose alterations of neuronal excitability caused by A β aggregates, other than synaptic plasticity studies (LTP/LTD) well known from the literature.

To study the molecular background of absence epileptogenesis, with special attention to the background of the change of the excitability of thalamo-cortical neurons and the role of inflammatory processes.

CONCLUSIONS

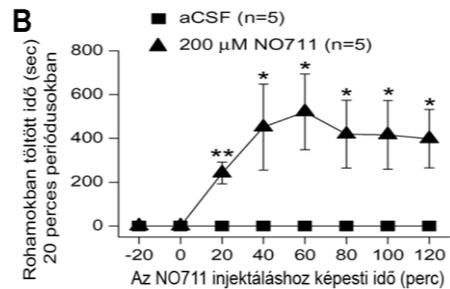
According to our experimental data it seems to be clearly proven that the continuously changing aggregate profile of β -amyloid solutions has different functional effects according to the degree of aggregation. Early aggregates are excitatoric, while later (larger) aggregates are inhibitory. This is likely to be due to the different targets of the oligomers dominating a certain solution. According to the effect on the pSpike this could even be the action potential generating site of the neurons. One of the target areas of the β -amyloid oligomers to be examined in the future is the thalamo-cortical network, involved in the generation of absence epileptic seizures, where even GABA receptors could be targets. The inflammatory responses that can enhance the number of absence epileptic seizures could also be considered targets of the A β . This is pointed out by the overlapping of the altered expression of proteins caused by inflammation and proteins known from the literature of Alzheimer's disease in the thalamo-cortical system. According to our data both in terms of cellular localization and in localization in the brain important new A β targets can be highlighted in the thalamo-cortical system. Results summarised in this thesis open a new path for future research in the directions cited above.

MATERIALS AND METHODS

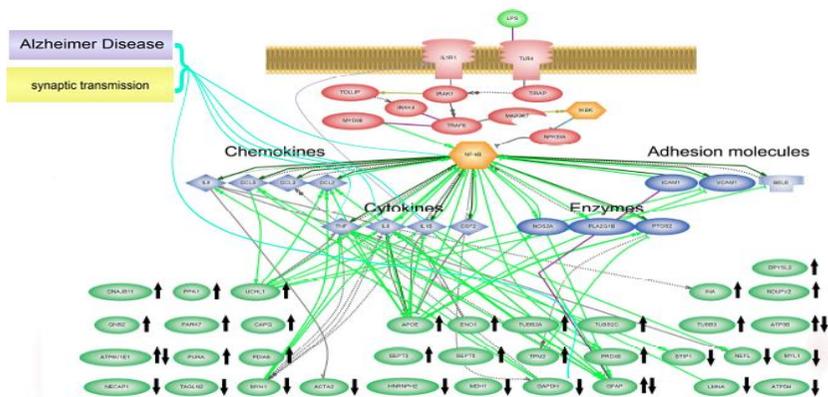
Preparation of homogeneously aggregated solutions

Preparation of monomeric amyloid by dissolving in 100% hexafluoroisopropanol (HFIP), freezing in liquid nitrogen, and lyophilization.

6. Blockage of the GAT-1 transporter in the VB of Wistar rats (NO-711 local treatment) increased ambient GABA levels, causing an enhanced tonic inhibition, which led to the appearance of absence seizures. The effect of NO-711 could be avoided by pretreatment with ETX.



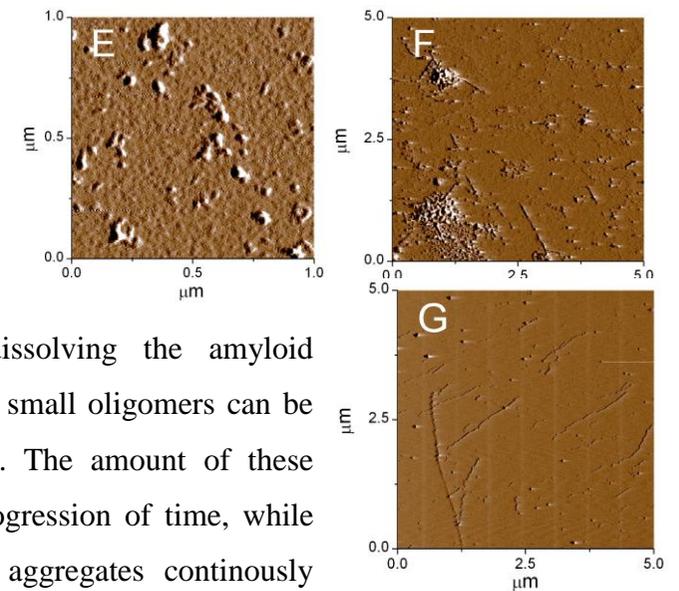
7. The pro-absence epileptic effect of LPS treatment links to a common neuro-immunological pathway with the pathway behind the



neuro-immunological processes of Alzheimer's disease according to literature. Both in the case of thalamus and cortex we experienced a change of the amount of the proteins belonging to the NFκB signal pathway, and its regulatory proteins.

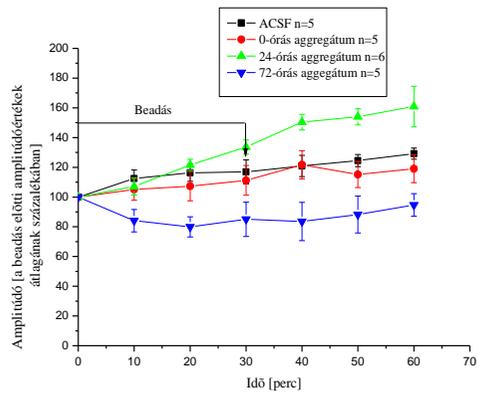
RESULTS

1. The aggregate composition changes with the time of incubation in terms of both morphology and size of aggregates.



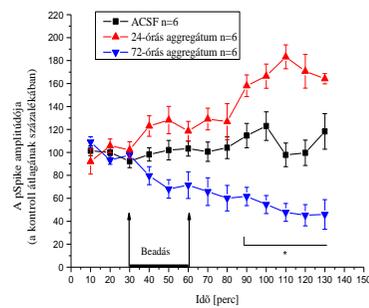
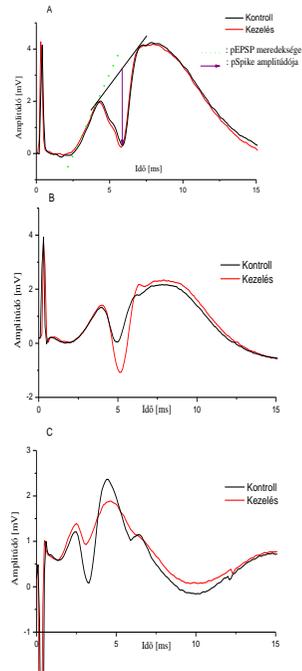
Immediately after dissolving the amyloid mostly monomers and small oligomers can be found in the solution. The amount of these decreases with the progression of time, while the amount of large aggregates continuously increases. The amount of large and aspecific aggregates reaches its maximum after 24 hours. Later on the amount of these also starts to decrease. In the 72 hours old solution the fibrillary amyloid is becoming dominant, and much less amorphous aggregates can be observed

2. Smaller aggregates caused an increase of firing of neurons, the solution containing partly large aggregates and partly fibrillary structures caused a decrease in excitability. Information gained about the facilitative interneurons and their synapses using paired pulse facilitation paradigm revealed that the changes of the amplitude of the population spikes in not due to the alterations of their facilitative inputs. The phenomena observed was not accompanied by a change of the pEPSP slope, suggesting that the inputs of the cells involved and the EPSP capability of their synapses were



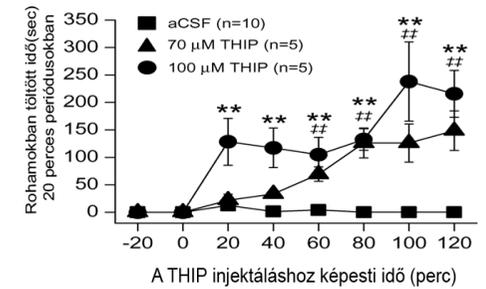
unaltered. The results of the control studies revealed that the urethane anesthesia itself altered the population firing. During the recording the responses gradually increased with time. This effect could decrease or mask the changes of excitability, so the experiments should be repeated in awake, behaving animals.

3. Experiments ran with awake animals have proven that the observed changes were really caused by the different aggregates. In this case the control (ACSF) recording was much more stable, the amplitude of the responses did not change significantly with time. Besides the effect of the aggregates was more dramatic than in experiments ran in urethane anesthesia. This is most likely due to the interaction of the anesthetic and the examined compound and of course the lack of it in awake condition. Similarly to the

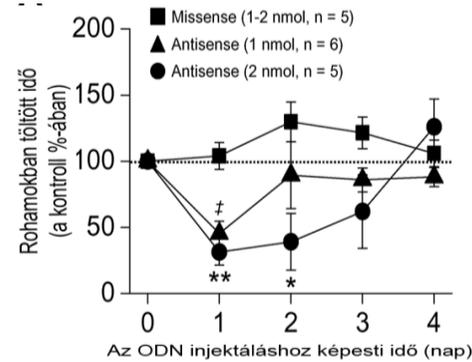


experiments in anesthetised animals, the slope of the pEPSP was also unaltered.

4. Pharmacological enhancement of tonic GABA_A inhibition in the VB (by local THIP treatment) is enough by itself to evoke absence epilepsy in behaving Wistar animals. This effect can be blocked by ETX pre-treatment.



5. A decrease of GABA_A δ subunit expression in the VB of GAERS rats prevents absence seizures.



The pathological increase in tonic GABA_A inhibition is necessary for the development of absence epilepsy. The effect of the gene silencing reached its maximum 24 hours after the injection of antisense ODNs, presumably this was the time required for the decrease of the amount of the receptor molecules in the cell membrane.