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Different Forms of the Amyloid β Peptide Affect Differentially the Electrical Activity of Cultured Neuronal Networks

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The amyloid β peptide (Aβ) has a central role in the pathology of the Alzheimer's disease. A number of cytotoxic effects have been attributed to it. There are several recent reports about the impact of Aβ on electrophysiological events in neurons. In a previous study we have shown that Aβ affects the electrical activity of cultured neuronal networks. In this work we compare the effects of different Aβ forms — $Aβ_{1-42}$, $Aβ_{1-40}$, and the fragment $Aβ_{25-35}$. The obtained results show that $Aβ_{1-40}$ affects the electrical activity of the neurons differently compared to $Aβ_{1-42}$ and $Aβ_{25-35}$.

Key words: Alzheimer's disease, amyloid β peptide forms, electrical activity of neurons.

Introduction

One of the morphological hallmarks of Alzheimer's disease are the senile plaques. Their core is built up from insoluble Aβ aggregates and is surrounded with degenerated neurons. For a long time it was assumed that it is these aggregated Aβ fibrils that are neurotoxic [3]. Numerous contradictory data stemming from research on Alzheimer's disease raised the question whether the aggregated Aβ fibrils have a central role or are only an element in the etiology of the disease.

It is known that Aβ is derived from its precursor protein as monomers which then aggregate in dimers, oligomers and fibrils, which can precipitate to form the core of the senile plaques. The location where these aggregation steps take place is unknown. A hypothesis postulates that the first aggregation steps occur intracellularly, because of the possibility to attain higher Aβ concentrations within cell compartments. Thereafter Aβ oligomers could be secreted out in the extracellular space where they act as an aggregation core.

There are a lot of controversial reports regarding the toxicity of Aβ in different aggregation states. Some authors attribute toxicity to monomeric Aβ species [10],

while others point out Aβ aggregates such as dimers [9], Aβ derived diffusible ligands [2], oligomers and fibrils as the carrier of toxicity [4].

The two basic endogenic forms of the Aβ monomer differ in the length of their amino-acid sequence. In the non-Alzheimer's brain the main form is $A\beta_{1-40}$ (ca. 90%) and some $A\beta_{1-42}$ is also secreted (ca. 10%). In the diseased brain this ratio switches so that $A\beta_{1-42}$ prevails, and this is the form found predominantly in the core of the senile plaques.

In a previous study we have reported that soluble Aβ inhibits the electrical activity of cultured neuronal networks in a concentration-dependent manner [6]. The aim of the present study is to compare the effects of the two forms — $A\beta_{1-42}$, $A\beta_{1-40}$, and the biologically active fragment $A\beta_{25-35}$.

Material and Methods

The effects of $A\beta_{25-35}$, $A\beta_{1-40}$ and $A\beta_{1-42}$ on the electrical activity of neuronal networks (frontal cortex cell culture from mouse embryos seeded on microelectrode arrays) were studied employing a neurotoxicity assay system described previously [5]. To ensure that in the employed solutions $A\beta_{(25-35;\ 1-40;\ 1-42)}$ are in their monomeric (soluble) form, we adopted a modification of the methods described by Butterfield et al. [1] and Zagorski et al. [12]. Individual networks were used for treatment with one form of the Aβ peptide.

Results and Discussion

It has been shown that the sequence from amino acid 25 to 35 carries the biological activity of Aβ [11]. Interestingly, although this sequence is present in both major endogenous forms of Aβ, they have different biological effects and show clear pharmacological differences [8]. Together with the switch in the ratio between $A\beta_{1-42}$ and $A\beta_{1-40}$ in healthy and diseased brain this guided us to compare their effect on the electrical activity of cultured neuronal networks.

In preliminary studies we could see that $A\beta_{25-35}$ has the strongest inhibitory effect, followed by $A\beta_{1-42}$ and $A\beta_{1-40}$ [7]. It was interesting to observe that during the incubation with $A\beta_{1-40}$ the electrical activity of the neurons tended to recover from the inhibitory effect.

In the present study we performed a set of experiments to verify our preliminary results and tested the Aβ-peptides and the Aβ-fragment at the most effective concentration — 50 μM.

The results show that $A\beta_{25-35}$ and $A\beta_{1-42}$ inhibit reversibly the electrical activity of the neurons (Fig. 1 and 2). The effect of $A\beta_{1-40}$ differs from these of the other two peptides. We observed initial inhibition followed by recovery to almost native activity (Fig. 3).

The difference between the action of the three peptides raises the question — are they acting through the same or through different mechanisms/sites? If they act through the same site it could be the difference in their structure that causes differences in their binding affinities, or steric factors become involved, thus resulting in differential effects. The structure of $A\beta_{1-42}$ could favor the stabilization of the hypothetic binding. If they act through different sites the mechanism of $A\beta_{1-40}$ action could implicate a feedback which alleviates the initial inhibition.

The exact mode of action of the tested Aβ peptides remains unclear and presents a challenge for further studies.

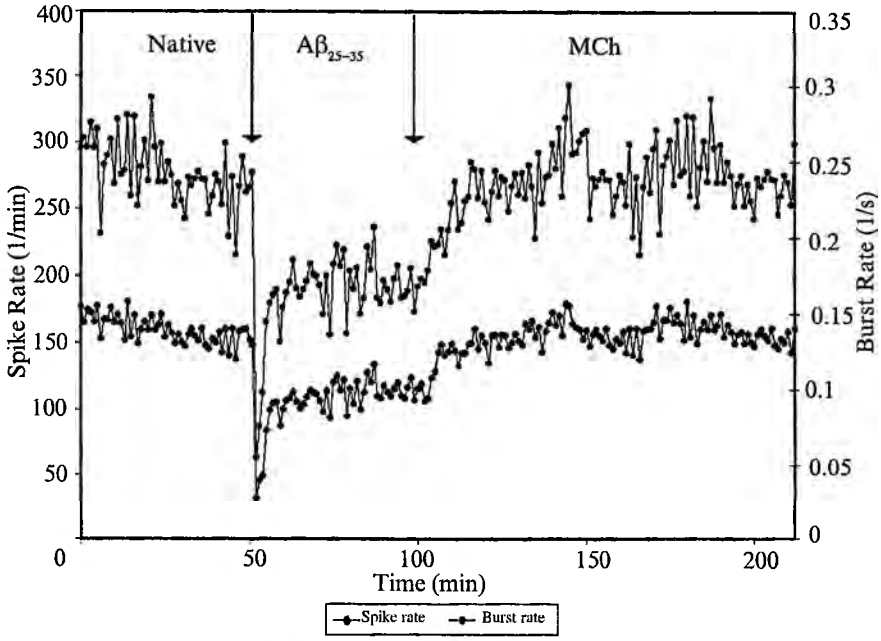

Fig. 1. Effect of 50 μM $A\beta_{25-35}$ on the spike and burst rates of a neuronal network. Burst rate is extracted using analog spike integration and is presented as bursts per second. Filled circles — spike rate; open circles — burst rate. Arrows indicate the time of onset of treatment of the network. Native — native electrical activity of the network; $A\beta_{25-35}$ — treatment with 50 μM $A\beta_{25-35}$; MCh — medium change, incubating medium replaced with fresh conditioned medium, lacking Aβ

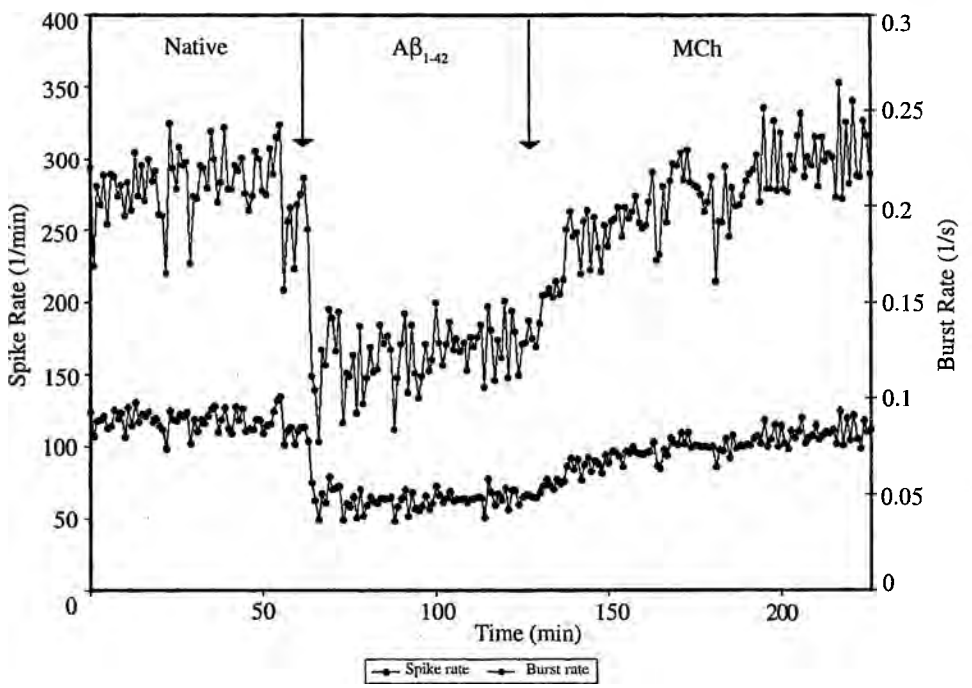

Fig. 2. Effect of 50 μM $A\beta_{1-42}$ on the spike and burst rates of a neuronal network. For details see Fig. 1

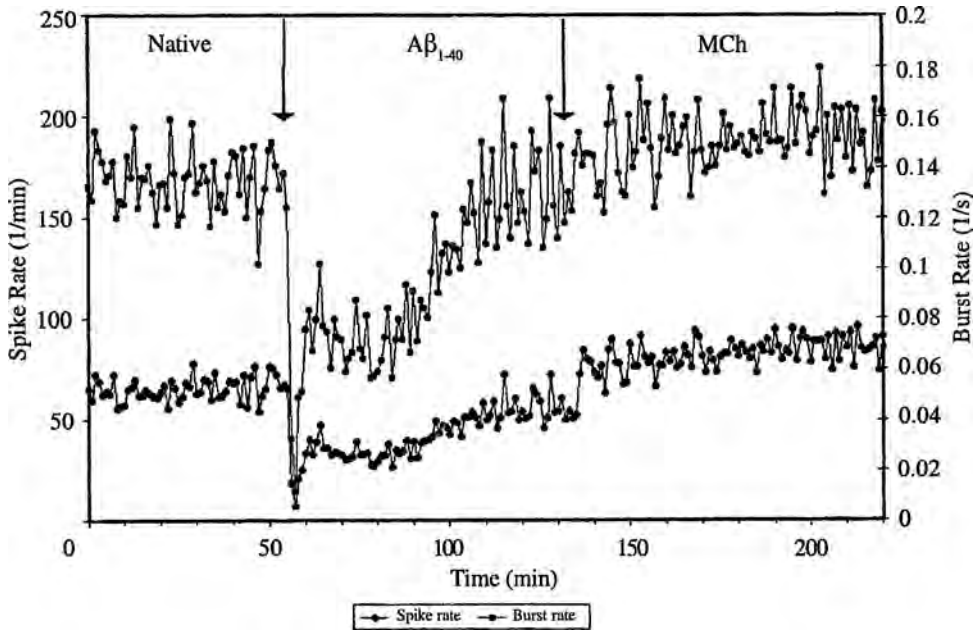

Fig. 3. Effect of 50 μM $A\beta_{1-40}$ on the spike and burst rates of a neuronal network. For details see Fig. 1

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