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Studies on the Effects of the Amyloidogenic Aβ-Peptide on the Electrical Activity of Neuronal Networks Cultured on Microelectrode Arrays

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The β-amyloid peptide (Aβ) is an amyloidogenic metabolic product of the amyloid precursor protein. It can aggregate into fibrils and larger structures, constituting the core of the amyloid plaques, characteristic of Alzheimer's disease. Soluble Aβ has been reported to cause peroxidation of membrane lipids, impairment of cholinergic neuronal function and damage of neuronal cell signalling. These led us to study the impact of soluble Aβ on the electrical activity of neurons cultured on microelectrode arrays. We found that low concentrations (nanomolar to micromolar) of soluble $A\beta_{25-35}$ lower both the spike and burst rates of neuronal networks. The effect is rapid, concentration-dependent, reversible and region-specific. On the basis of the obtained results we can now hypothesize that the impairment of neuronal electrical activity by the amyloidogenic Aβ peptide may induce the loss of communication between neurons and be one of the causes for Alzheimer's disease.

Key words: β-amyloid peptide, electrical activity of neurons, neuronal networks.

Introduction

Alzheimer's disease (AD) is characterized pathologically by extracellular deposition of senile amyloid plaques, the principle constituent of which is the β-amyloid peptide Aβ is a 39-43-amino acid peptide, which is one of the metabolic products of the amyloid precursor protein (βAPP). The normal metabolic processing of βAPP generates both amyloidogenic and non-amyloidogenic products [19].

The sequential cleavage of βAPP at the NH_2-end of the Aβ sequence by the newly identified β-secretase [10] and by putative γ-secretases [25] at the C-terminus yields the intact Aβ segment. The Aβ peptide is recognized for its ability to self-aggregate into large fibrils and β-sheet secondary structures, comprising the core of the amyloid

plaques. Many studies support the notion that neurodegeneration in AD is driven by amyloid deposits and/or fibrils [12]. This led to the "amyloid cascade hypothesis", formulated by Hardy and Higgins [6]. They state that Aβ "precipitates to form amyloid and, in turn, causes neurofibrillary tangles and cell death". Despite the abundance of data in favor of this hypothesis [16, 23], the pathogenic role of amyloid deposits themselves is still a controversial issue. Most problematic is the weak correlation between fibrillar amyloid deposition and levels of neurological dysfunction [26]. Furthermore, amyloid deposits are often found at a distance from sites of neuronal loss. New evidence suggests that the best pathological correlate of dementia is the loss of synaptic terminals, which correlates poorly with the amount of amyloid deposition [15, 20]. These recent findings brought about a shift in the conceptions based on the historical view, that fibrillar Aβ is the primary candidate as the neurotoxic element in Alzheimer's disease. Increasing evidence is emerging about the strong correlation between the severity of AD and the soluble amyloid components [18].

Soluble Aβ is secreted by a variety of cells and has been found in human brain and in CSF. An interesting observation is that in brains of AD patients the level of soluble Ab is higher than in controls, while in CSF soluble Aβ appears to decrease with increasing severity of AD [13, 8]. Soluble Aβ exhibits biological actions at low (nanomolar) concentrations: it induces peroxidation of membrane lipids of cultured neurons or isolated synaptosomes [11]; soluble Aβ induced oxidative stress results in impaired glucose and glutamate transport in synaptosomes [17]; soluble Aβ affects the cholinergic neuronal function of cultured neurons [21]. It has also been found that at low concentrations Aβ exhibited neurotoxicity, impairing neuronal cell signalling, but had no cytotoxic effects, since it did not affect neuronal survival [9].

During the last two years the latter data led to the initiation of research on the electrophysiological impact of small protofibrillar and soluble Aβ forms. It was reported that Aβ oligomers, termed ADDLs [14] are cytotoxic, killing hippocampal neurons at nanomolar concentrations by chronic (24 hour) application. ADDLs elicited a rapid (within 45 min of application) inhibition of long term potentiation in tissue slices, despite retention of the capacity for evoked action potentials and prior to the onset of cell death. It was also found that NMDA receptor antagonists did not inhibit ADDL toxicity. Similarly, 200 nm Aβ-derived protofibrils elicited rapid electrophysiological changes, inducing membrane depolarization and increased EPSPs and action potentials [7]. Another Aβ-containing toxic moiety is the CT_{105} βAPP-derived fragment, which was shown to induce a depression of Parallel fibre — Purkinje cell synaptic transmission in cerebellar slices [5]. These authors examined in parallel the effect of the Aβ sequences 1-16, 25-35 and 1-42 and found that CT_{105} and $Aβ_{25-35}$ were most potent in inducing depression of AMPA-receptor-mediated synaptic transmission through a combination of pre- and postsynaptic effects, indicating that Aβ fragments can modulate AMPA-mediated synaptic transmission. It has also been suggested that the coupling between M1 muscarinic acetylcholine receptors and G-proteins can be impaired by β-amyloids, and M1-selective muscarinic agonists have therapeutic potential.

In an attempt to address the question of the effect of soluble Aβ on the electrical activity of nerve cells in a more direct manner we employed the model system created by G r o s s [3, 4], using neurons grown on microelectrode arrays and termed "Neuronal networks cultured on microelectrode arrays". Since many studies show that the fragment of Aβ enclosing amino acids 25-35 is most effective in generating biological responses we studied the effects of soluble $Aβ_{25-35}$ on the electrical activity of spinal cord, whole cortex and frontal cortex-derived neuronal networks.

Materials and Methods

Spinal cord and frontal cortex tissues dissociated from 14-15-day and 17-day-old mouse embryos, respectively, were cultured on microelectrode arrays at concentrations of 250 000 cells/0.5 ml. The cultures were mainained at 37 °C in an atmosphere of 90% air and 10% CO_2. The networks develop spontaneous activity at about 5-7 days, stabilize at 3 weeks and remain active for several months [4].

The 64-electrode arrays were made using standard photoetching techniques. Recording of the data was performed with the Multichannel Acquisition Processor System, a computer-controlled 64-channel amplifier system commercially available from Plexon, Inc. (Dalas, TX). Action potential (spike) data from active channels were integrated. Analog spike integration was used as a major scheme for extraction of burst activity from single channel spike patterns [2].

Results and Discussion

$A\beta_{25-35}$ reduces the electrical activity of spinal cord neuronal networks in a reproducible and concentration-dependent manner, lowering both the spike and the burst rates of the networks.

The effect is evident already at 25 nM Aβ and evolves within the first 3 minutes of addition to the network. An almost complete shut-off occurs at 50 μM Aβ. Lowering the concentration of Aβ by medium change resulted in a recovery of electrical activity.

The effect of $A\beta_{25-35}$ was also tested on cortex-derived networks of the same age. Comparing the influence of Aβ on these two tissues we found that cortical cultures are affected to a significantly lesser extent (Fig. 1). However, they also show reproducibly a recovery of activity on reducing of Aβ concentration by medium changes. The differential effect of Aβ on spinal cord and frontal- or whole-cortex cultures may reflect a region specific action of the Aβ peptide, reflecting the presence/abundance of different transmitter systems in these central nervous system (CNS) regions.

It is worth noting that after the initial reduction of electrical activity between 25 and 75 nM Aβ there is an activation stage between 100 nM and 1 μM Aβ. Similar stimulation of other biological activities at these intermediate Aβ concentrations have been described in other model systems. Low concentrations have a neurotrophic effect, but high concentrations are neurotoxic to cultures of rat hippocampal neurons [27]. Low concentrations of Aβ have opposing effects on cellular ionic activity compared to higher ones [1]. Similarly, low concentrations stimulate, while high concentrations inhibit neurite outgrowth in primary cultures [22]. The observed activation of network activity at intermediate Aβ concentrations may reflect a naturally occurring mechanism for physiological control, brought about by transient physiological elevations of Aβ concentration. This can be accomplished by the neurons through regulation of the proteolytic breakdown of APP.

Treatment of the networks with Aβ did not affect action potential amplitude and shape, implying that cellular energy metabolism was not influenced. Therefore it can be expected that Aβ acts at the synapse itself.

It has been reported that soluble Aβ can induce peroxidation of membrane lipids of cultured neurons or isolated synaptosomes [11, 17]. To check whether the effect of Aβ on the electrical activity of neuronal networks is due to lipid peroxidation, i.e. a general, non-specific effect upon the networks, we compared the ef-

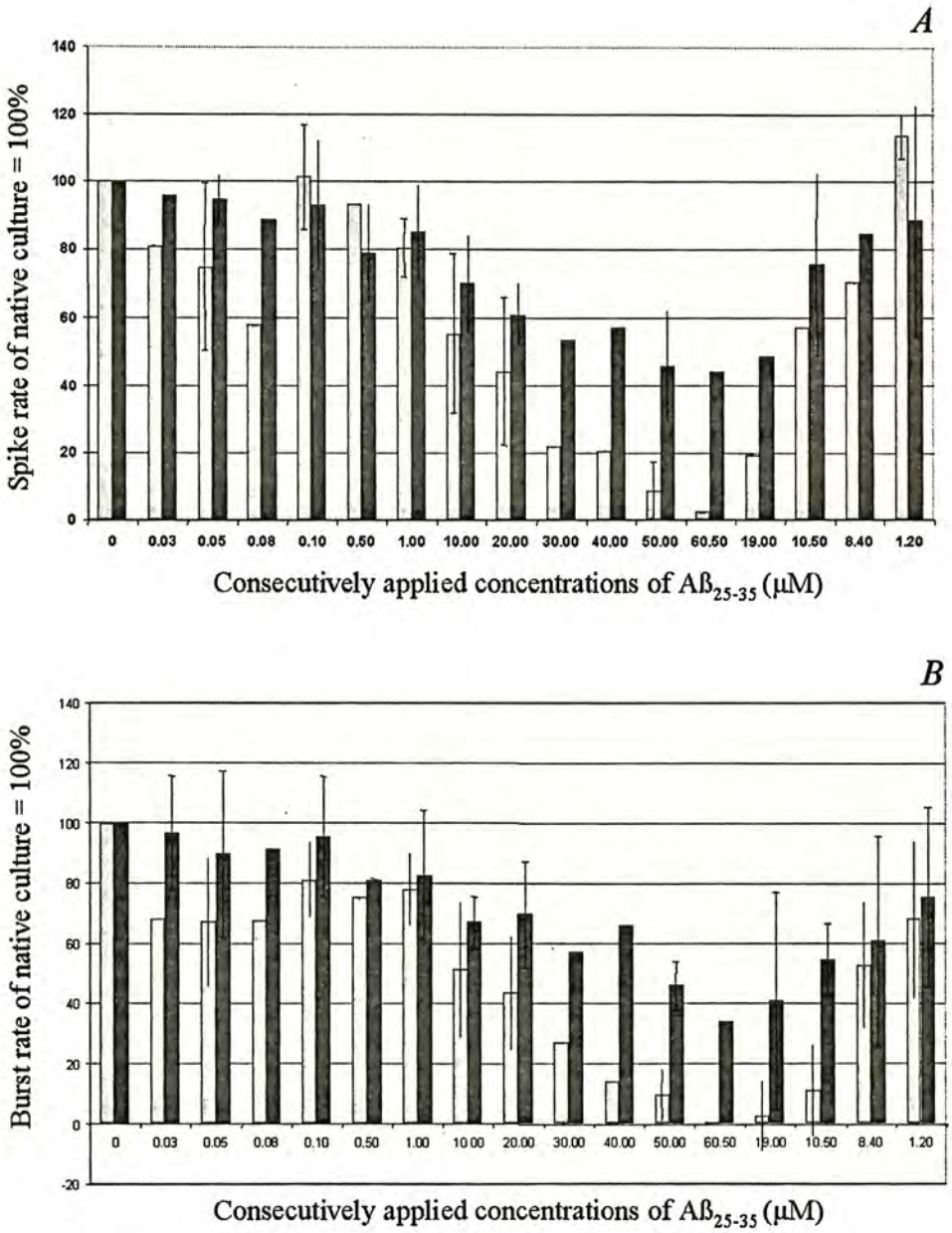

Fig. 1. Comparison of the effects of $A\beta_{25-35}$ on the spike (*A*) and on the burst (*B*) rates of spinal cord (grey columns) and whole cortex (black columns) networks; bars indicate SD when five or more experiments are analyzed

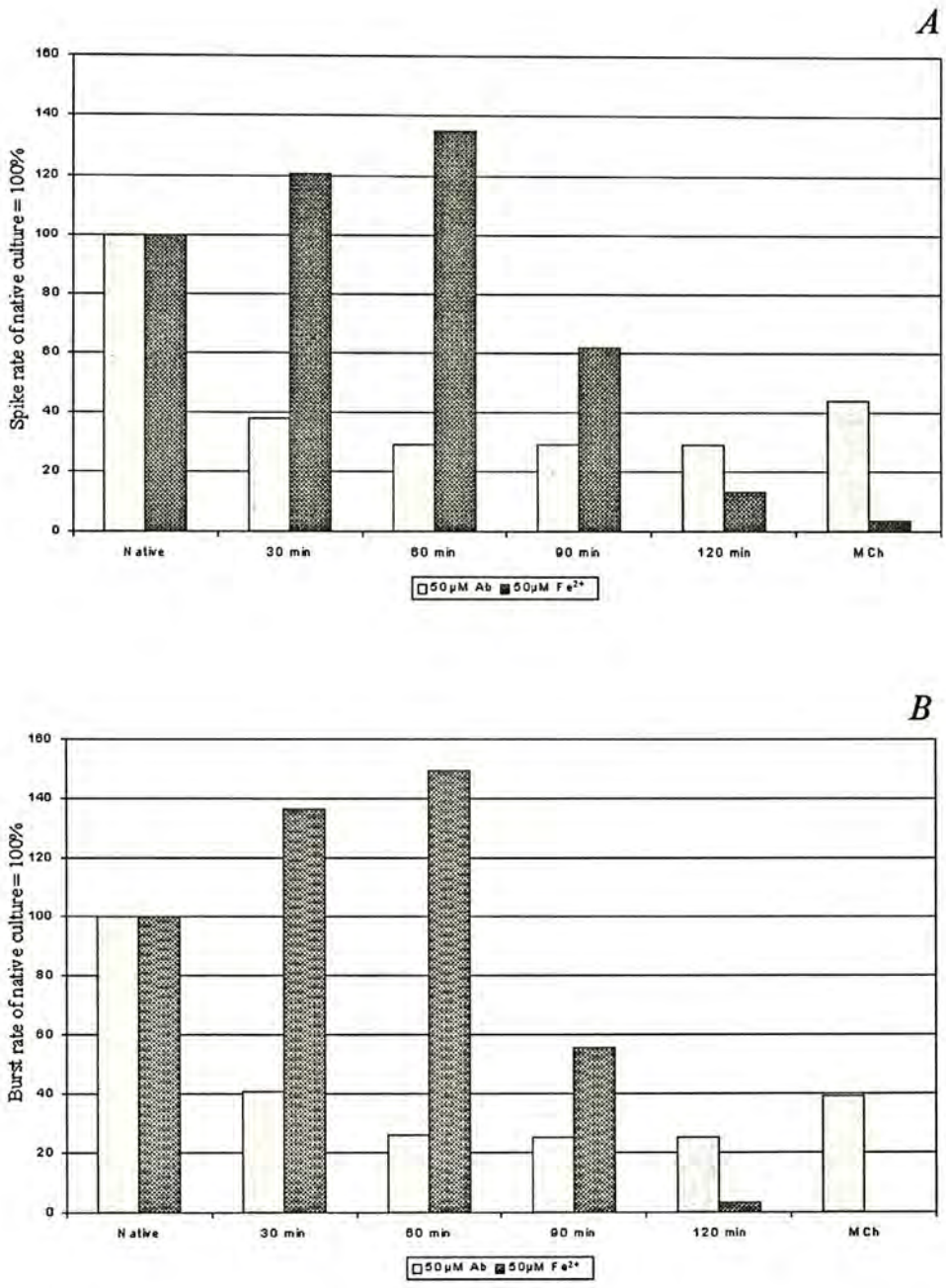

Fig. 2. Comparison of the effects of 50 μM $A\beta_{25-35}$ and 50 μM Fe^{2+} on the spike (*A*) and on the burst (*B*) rates of frontal cortex networks, treated with the agents for 120 min, followed by medium change - MCh

fects of Aβ to that of Fe^{2+} ions, an agent known to induce lipid peroxidation [17]. Treatment of neuronal networks with 50 μM $FeSO_4$ (Fig. 2) for various periods of time also brought about a decrease and eventually to cessation of electrical activity. However, its effect shows quite a different time dependence. Its action is delayed, as compared to the immediate action of Aβ, and in addition the effect of Fe^{2+} ions is not reversible.

Our observations that the effects of Aβ are very fast (3 min onset), concentration dependent (attaining a plateau at each concentration), reversible, and CNS region specific can be taken as proof for the specificity of its action. Our data provide direct evidence that Aβ influences the electrical activity of neuronal cells. The observed neurotoxicity of soluble Aβ is consistent with the thesis of a "plaque-free pathology" of Alzheimer's disease [12] discussed above.

The lack of effect of Aβ on action potential amplitude and shape, the rapidity of the response of neuronal networks to application and removal of Aβ, and the specificity towards different CNS regions imply that: a) Aβ exerts its action directly at the synaptic site; b) its action is most probably receptor-mediated.

In search of the transmitter system affected by soluble Aβ we performed preliminary experiments employing known antagonists to neurotransmitter receptors.

The first set of experiments was performed to compare the action of Aβ with that of bicuculline (BCC) and strychnine, the antagonists to the receptors of the inhibitory neurotransmitters $GABA_A$ and glycine respectively.

The effects on the electrical activity of neuronal networks of these substances were described by a number of characteristics, the major ones of which are the spike rate (SR), the burst rate (BR), the Cv net of burst rate (CVnBR) and interburst spiking

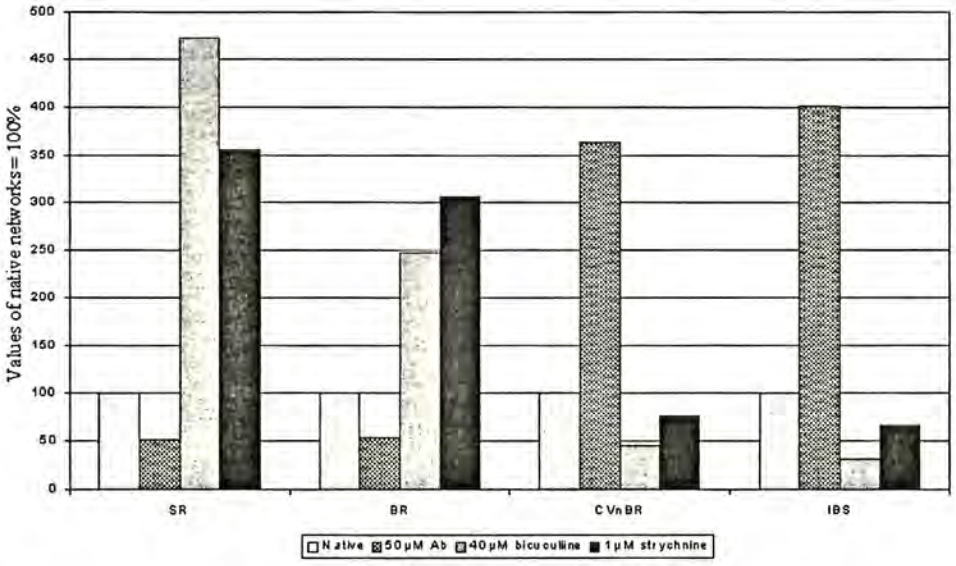

Fig. 3. Comparison of the effects of Aβ, bicuculline and strychnine on the major characteristics of neuronal networks.
SR — spike rate; BR — burst rate; CVnBR — CV net of burst rates; IBS - % of interburst spikes

(IBS). SR and BR are a direct measure of a networks activity. CVnBR reflects the synchronization of the bursts between the different units, smaller values pointing to a higher degree of synchronization. IBS is a measure of random spikes, not included in the bursts.

The effects of Aβ in the absence (or in the presence, data not shown) of either antagonists are in all instances opposite to those of the antagonists (Fig. 3). Blocking of the $GABA_A$-receptor by bicuculline, or of the glycine-receptor by strychnine result in highly synchronized bursting of the networks and an almost complete disappearance of interburst spiking. Addition of Aβ to the network causes reappearance of IBS, decrease of the number of spikes in the bursts, shortening of the burst duration and gradual dissolution of bursts and synchronization of bursting. This is consistent with Aβ acting as an agonist on one of the receptors for inhibitory transmitters.

On the basis of the results obtained thus far we can now propose the hypothesis that the reduction of electrical activity, and thus — the impairment of communication between neurons, brought about by the amyloidogenic Aβ peptide, is one of the causes for Alzheimer's disease. This opens up a new trend in the studies on the effects of the soluble Aβ peptide.

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