Alzheimer's β -Amyloid-Induced Depolarization of Skeletal Muscle Fibers: Implications for Motor Dysfunctions in Dementia

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Key Words

Alzheimer's disease • β -amyloid peptide • Depolarization • Gait disorders • Muscle

Abstract

Numerous findings obtained over the last decades suggest that accumulation of β -amyloid peptide (β AP) plays the central role in the pathogenesis of Alzheimer's disease. It is well established that β AP has wide range of toxic effects on neurons *in vitro* and *in vivo*, however the influence of β AP in the periphery and on various other types of excitable tissues, eg. skeletal muscle cells, is almost unknown despite the many non-cognitive and other extra-neuronal symptoms associated with Alzheimer's dementia. Here we utilized conventional electrophysiological technique to investigate the effects and mechanisms of β AP action on the resting membrane potential of frog skeletal muscle fibers. β AP in the range of concentrations from 10^{-6} to 10^{-8} M produced slow, significant, reversible depolarization of muscle fiber membranes. The impact developed and was washed out faster at higher concentrations of β AP (10^{-6} – 10^{-7} M). The effect of β AP was completely absent when applied in Na^+ -free Tris⁺

solutions. β AP-mediated depolarization was also prevented by tetrodotoxin (10^{-5} M) pre-treatment and rescued by tetrodotoxin after-treatment. These findings suggest that β AP-induced depolarization of skeletal muscle plasma membranes can significantly disturb the functioning of skeletal muscles and therefore contribute to motor dysfunction observed in Alzheimer's disease and other disorders associated with β AP accumulation.

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Introduction

β -amyloid (β AP) is a small peptide of 38-43 amino acids ($\beta\text{AP}_{1-38/1-43}$), which derives from the trans-membrane amyloid-precursor-protein (APP) via proteolytic reactions [1-3]. β AP is produced normally and can be found in plasma, cerebro-spinal fluid (CSF) and brain tissue of healthy people [4-7], but β AP over-production seems to be causative for Alzheimer's disease (AD) [2, 8]. β AP prones to form long insoluble fibrils, which appear to be a main component of amyloid deposits in the brain and some other tissues (skin, muscles, etc.)

of AD patients [1, 3, 6, 9]. Accumulation of β AP is observed in patients with other neuro-degenerative disorders (Parkinson's disease, Lewy body dementia, Down syndrome) and muscle-degenerative disease, eg. inclusion body myositis [1, 6, 8, 10, 11].

β AP both in soluble and fibrillar forms has wide range of neurotoxic effects *in vitro* and *in vivo*, including mitochondrial dysfunction [1, 3], intracellular calcium imbalance [12], impairment of synaptic transmission and use-dependent plasticity [13-16], generation of oxidative stress [17], modulation of ion transporting systems [18], etc. [3, 8]. These findings suggest crucial role of β AP in the pathogenesis of AD and other neurodegenerative diseases accompanied by β AP accumulation.

The effects of β AP on neuronal functions are widely investigated, but very little is known about its effects on another type of excitable cells – for instance, skeletal muscle fibers. Previously it was found that the levels of β AP₁₋₄₀ and β AP₁₋₄₂ are significantly increased in the skeletal muscles of AD patients [6]. The question about the effects of β AP on muscle fibers is of significant interest, because patients with AD, inclusion body myositis, as well as with other β AP-associated disorders often exhibit severe motor dysfunction [6, 19-21]. β AP-mediated disturbance of skeletal muscle functioning can serve as an important contributor to such symptoms. It is not surprising that Alzheimer's β AP may be responsible for gait disorders associated with dementia, in that AD has recently been considered to be a systemic disease with the most prominent pathology in the cognitive functions of the brain [12]: its toxic effect, therefore, may manifest not only in the central nervous system (CNS), but also in extra-neuronal tissues, including muscles.

Based on these observations and theories, here we investigated the influence of β AP (fragment 25-35: β AP₂₅₋₃₅) on the resting membrane potential of skeletal muscle cells in frog. We found that β AP has strong dose-dependent reversible depolarizing action on plasma membranes of skeletal muscle fibers. We have also revealed and describe the ionic mechanisms of this phenomenon.

Materials and Methods

Experiments were carried out on frog *Rana ridibunda* sartorius muscle preparation between October and February (during hibernation period). The experiments were done on amphibians, in that frogs are one of the most widely used animals in muscle electro-physiology, and generally recognized as a model to study the electro-genesis of skeletal muscle fibers.

Animals were stunned under ether anesthesia and were pithed in accordance with the European Communities Council Directive (86/609/EEC: November 24, 1986). Preparations were dissected and mounted to a recording chamber. Physiological saline solution (113 mM of NaCl, 2.5 mM of KCl, 2 mM of CaCl₂, 2 mM of MgCl₂, 2.4 mM of NaHCO₃) was continuously perfused over the preparation. Na⁺-free solution was prepared by equimolar substitution of NaCl with Tris⁺. pH of all solutions was adjusted to 7.3 at 20°C.

The 11-amino-acid fragment of β AP (β AP₂₅₋₃₅) and tetrodotoxin (TTX) were obtained commercially from Ascent Scientific Ltd. (UK), whereas all other reagents were purchased from Sigma-Aldrich (St.Louis, MO, USA). β AP₂₅₋₃₅ has the functional domain of full-length β AP required for both neurotrophic and neuro-toxic effects [13,14,22]. Peptides were dissolved in phosphate-buffered saline (PBS) and incubated for an hour to 'age' before direct use in the experiments.

The resting membrane potential of skeletal muscle fibers was measured using conventional electrophysiological technique. For each point of time, membrane potential was measured in extra-synaptic region (without miniature end-plate potentials) by impaling 20 or more fibers with glass microelectrodes during 3-4 minutes (2.5 mM of KCl, 5-10 M Ω).

The software Microcal Origin 5.0 was used for statistical analyses. All data are presented as mean $\pm$ S.E.M. (standard error of the mean), with statistical significance assessed by Student's *t*-test. The *P* value of less than 0.05 was considered as statistically significant difference.

Results

β AP₂₅₋₃₅ dose-dependently depolarizes skeletal muscle membrane

At control conditions membrane potential was 73.4 $\pm$ 0.7 mV (n=195, n: number of muscle fibers). Application of 10⁻⁶M of β AP₂₅₋₃₅ caused slow decrease in the membrane potential (42.4 $\pm$ 1.2 mV, n=175) at 45th minute of action. From this point, membrane potential remained stable at a significantly lower level (*P*<0.001). The depolarizing effect of β AP was reversible: 30min wash-out led to complete recovery of membrane potential to initial values (72.7 $\pm$ 1.8 mV, n=184, Figure 1A). The visual control under light microscopy (x 100 magnification) revealed no spontaneous contraction of muscle fibers before, during or after the application of β AP.

Lower levels of β AP (10⁻⁷M and 10⁻⁸M) were also tested. Both concentrations had similar effect, but they decreased membrane potential slower, and wash-out of the effect also was retarded. β AP at 10⁻⁷M reduced the membrane potential to 42.5 $\pm$ 1.7 mV (n=154, *P*<0.001 compared to control, t=75min, t: time). The wash-out of β AP (10⁻⁷M) until the restoration of membrane potential took 45 minutes (Figure 1B). Treatment of muscle with

Fig. 1. The effects of βAP_{25-35} at different concentrations on the resting membrane potential of skeletal muscle fibers: Figures represent the development of βAP_{25-35} depolarizing effect (the presence of βAP in perfusion solution is indicated by thick line, A: 10^{-6}M , B: 10^{-7}M , C: 10^{-8}M) and restoration of membrane potential under wash (shown as thin line). Abscissa line: time scale in minutes; ordinate line: average values of membrane potential in mV.

10^{-8}M of βAP caused even slower reduction of membrane potential (37.1 ± 1.1 mV, $n=146$, $P<0.001$ compared to control, $t=150\text{min}$, Figure 1C).

Thus, βAP_{25-35} in a range of concentrations from 10^{-6}M – 10^{-8}M caused strong, dose-dependent, reversible depolarizing action on the membrane potential of skeletal muscle fibers.

Effect of βAP_{25-35} on membrane potential depends on the presence of extra-cellular sodium ion (Na^+)

The mechanisms of βAP -mediated disturbance of muscle fiber membrane potential remained theoretical. Increased influx of Na^+ to sarcoplasm from the extra-cellular space may be a possible cause of strong membrane depolarization. At normal conditions, resting sodium-influx is very small: tens of times smaller than potassium (K^+) efflux.

To determine the role of Na^+ in βAP 's depolarizing action, perfusion solution in which Na^+ -ions were substituted with Tris^+ was used. Application of Tris^+ solution itself did not change the amplitude of membrane potential as measured during 45min period of action ($n=165$, $P>0.05$). Application of 10^{-6}M of βAP , co-administered with Tris^+ solution, did not produce any significant changes of membrane potential ($n=187$, $P>0.05$, Figure 2A).

Taken together, βAP -induced depolarization of muscle fiber membrane is apparently mediated by increase of Na^+ -influx.

TTX pre- or post-treatment abolishes βAP -induced membrane potential disturbances

Because βAP has been proposed to directly form non-selective ion-transporters in the membranes [23], the important matter whether βAP -induced Na^+ -influx flows through specific Na^+ -channels or non-selective βAP -pores remained to be elucidated. To address this question, TTX (selective blocker of Na^+ -channels) was used. Application of 10^{-5}M of TTX slightly increased the resting membrane potential from 74.6 ± 0.8 mV to 82.5 ± 0.9 mV ($n=175$, $P<0.05$). This hyper-polarization, evidently, indicates the removal of resting Na^+ -influx. Further application of 10^{-6}M of βAP in the presence of TTX did not produce any changes of membrane potential as measured during 80min of βAP action. Finally, 30min wash-out of muscles with Ringer-solution brought the membrane potential back to initial values (74.9 ± 1.3 mV, $P>0.05$, Figure 2B).

It has also been tested whether TTX enjoys the ability to eliminate βAP -induced depolarization when βAP is applied first. To evaluate this, 10^{-6}M of βAP was added to the perfusion solution and incubated until its depolarizing effect had completely developed (dropped from 74.8 ± 0.8 to 41.38 ± 1.0 mV, $n=167$, $P<0.05$). Further application of 10^{-5}M of TTX in the presence of βAP has led to complete recovery of membrane potential back to initial values (75.0 ± 1.1 mV, $n=175$, Figure 2C).

As such, both pre- and post-treatment of muscles

Fig. 2. The sodium nature of β AP₂₅₋₃₅-induced depolarization of skeletal muscle fibers: A: 10^{-6} M of β AP (indicated by thick line) had no effect in Na⁺-free Tris⁺ solution (Tris⁺). Pre- (B) and after- (C) treatment of muscle with Na⁺-channel blocker TTX (10^{-5} M) rescued the β AP-induced depolarization of muscle fibers. Abscissa line: time scale in minutes; ordinate line: average values of membrane potential in mV.

with TTX eliminate β AP-induced depolarization of fibers, indicating that β AP produces increased Na⁺-influx to sarcoplasm through TTX-sensitive Na⁺-channels. These results also prove that increased Na⁺-permeability of skeletal muscle membrane is not mediated by β AP-formed ion-channels, inasmuch as these channels are not blocked by TTX [18]. Of note, this non-selective channel-forming effect of β AP is reminiscent of the properties of pore-forming proteins, *eg.* perforin (the membrane attack complex of complement) [3].

Discussion

The mechanisms and consequences of β AP-induced disturbance of skeletal muscle membrane electro-genesis

Our studies have revealed the novel effect of β AP: strong depolarizing action on the plasma membranes of skeletal muscle cells in rest (without action potentials). We have also found that β AP-induced depolarization is mediated by increase of Na⁺-influx through Na⁺-channels. The mechanism by which β AP induces activation of Na⁺-channels is unknown. Considering rather long time scale of development of β AP effect (more than 30 min), we suggest indirect modulation of Na⁺-channels via some metabolic regulatory cascade(s). There are possibilities for the involvement of different membrane proteins (nicotinic receptors, nicotinic receptors, G-protein coupled receptors, Na⁺/K⁺-ATPases and other ion-pumps, etc.), cyclic nucleotide systems (cAMP, cGMP), reactive oxygen species (ROS)-generating system, intracellular calcium imbalance, etc. [1-3, 12, 17, 18, 24, 25]. Besides Na⁺ channels, direct and indirect effects of β AP on several active and passive transport systems may also result in depolarization of plasma membranes, leading to β AP-induced activation of voltage-dependent Na⁺-channels. Finally, direct or ROS-mediated influence of β AP on the lipids of skeletal muscle plasma membrane may affect the functioning of membrane proteins, including Na⁺-channels [3, 26, 27].

Interestingly, the varying concentration of β AP (10^{-6} - 10^{-8} M) changed the duration of effect development only, but not the intensity of depolarization: higher concentration of β AP caused more rapid, but not more significant effect on membrane potential. This phenomenon certifies that the mechanism of β AP-induced membrane depolarization is saturated in the range of concentrations used, suggesting the very high affinity and low speed of interaction between β AP and effector molecules.

Surprisingly, β AP-induced depolarization did not trigger muscle contractions, which can be explained by the adaptation of muscle membrane to the neuro-toxic peptide, inasmuch as the slow time-course (tens of minutes) of developing depolarization allows myo-fibers to accommodate to creeping changes in the membrane potential. Nevertheless, β AP-induced depolarization of skeletal muscle cell membrane may significantly alter the neuro-muscular transmission and muscle contractility. In agreement with the voltage-current relationship, drop of membrane potential of muscle fiber to about -40 mV leads

to significant decrease of the end-plate potential amplitude [28]. This effect reduces the reliability of neuro-muscular transmission, which can be measured quantitatively by safety factor [29]. Moreover, such a strong depolarization that we found would significantly impair all other functions of skeletal muscle fibers, including intra-cellular ionic homeostasis, ion transport across the cell membrane, excitation-contraction coupling, etc. All these impairments may manifest clinically as gait problems in AD patients, as confirmed by epidemiological studies [19-21].

Consistency with previous studies

Garcia and colleagues [22] found that βAP_{25-35} in similar concentration range (0.1-10 μM) has effects on neither neuro-muscular transmission, nor membrane potential of skeletal muscle fibers in rat. However, that study was performed with the use of μ -conotoxin (specific inhibitor of muscle-type Na^+ -channels) to block muscle fiber action potentials. Evidently, this fact clearly explains the difference in the obtained results, because we have also not found any βAP -induced depolarization after blocking the Na^+ -channels in muscle. Absence of βAP effects on neuro-transmitter release shown in this study suggests that soluble βAP_{25-35} does not depolarize the motor nerve terminal membranes.

In another study, Ashenafi et al. [13] showed that 800-1200nM of βAP_{25-35} produces an unpredictable effect, membrane depolarization and/or hyper-polarization, on neurons of baso-lateral amygdaloid complex in slices. This effect was blocked by TTX or application of blockers of GABA-ergic and glutamatergic receptors together. Authors suggested that the revealed changes of membrane potential in studied neurons are driven by βAP -induced modulation of synaptic activity, but not by changes of voltage-gated sodium permeability of the membrane. Comparing their results with ours, we suggest that βAP_{25-35} -induced Na^+ -channel-dependent depolarization of plasma membrane is a phenomenon specific to muscles (skeletal muscles).

Clinical implications

Most AD patients suffer from motor dysfunction, such as gait and posture disorders, apraxia, oculomotor disturbances, bradykinesia, tremor, etc. [19-21, 30]. There is accumulating evidence suggesting that motor impairment can be a characteristic feature of not only late stages of the disease, but also of early AD pathology [19]. The phenomenon of βAP -induced skeletal muscle impairment can serve as an important contributor to motor dysfunction in AD.

Our results raise the question whether βAP indeed induces strong depolarization of skeletal muscle cells in AD and/or other βAP -associated disorders or not? Even if this phenomenon is occurred *in vitro*, it may not so clearly manifest clinically. The first reason is that the lowest concentration of βAP_{25-35} that we used in our experiments (ie. 10nM) is much higher than the serum levels of βAP_{1-40} and βAP_{1-42} of AD patients (ie. 0.07 and 0.02nM, respectively [7]). Apart from these, some endogenous βAP -binding compounds (eg. trans-thyretin, albumin, transferrin, etc. [31-33]) and/or proteases that that degrade βAP (eg. insulin-degrading enzyme and neprilysin [34]) may effectively prevent this type of βAP -mediated toxicity.

However, we cannot totally exclude βAP -mediated membrane potential disturbance *in vivo*. In natural conditions muscles are exposed to βAP for much longer times than fibers that have been studied in our experiments. Furthermore, even if βAP produces *in vivo* permanent depolarization of skeletal muscle cells of only few millivolts via increase of sodium permeability, it may be enough to induce severe ionic imbalance in muscle fibers, change the contractility properties of muscles and affect neuro-muscular transmission. This manuscript is the first to demonstrate evidence that βAP itself can impair the functioning of skeletal muscles that represents the mechanism for motor dysfunctions in AD and other βAP -related gait disorders.

Conclusion

This is the first study to demonstrate a novel type of βAP -induced cyto-toxicity: the reversible depolarization of skeletal muscle fiber plasma membranes at rest. We have also found that βAP -induced depolarization is driven by increased Na^+ -influx to muscle fibers through TTX-sensitive Na^+ -channels. The exact molecular mechanism of the revealed phenomenon, however, is yet unknown. We suggest that βAP -induced disturbance of skeletal muscle membrane potential may contribute to motor dysfunctions observed in AD and other disorders associated with βAP accumulation.

Acknowledgements

This study was supported by RFBR (07-04-01331), RF President Grant (NSh-3368.2008.4), and by *Asklepios-Med Bt*.

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