Impaired electro-genesis in skeletal muscle fibers of transgenic Alzheimer mice

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ABSTRACT

Alzheimer’s disease (AD) is characterized by memory decline, but is often associated with non-cognitive symptoms, including muscular dysfunction. In the majority of cases these motor disturbances are seen when other neuro-degenerative disorders such as Parkinson’s disease overlap dementia, however these can also be directly related to AD itself. Although the patho-mechanism remains largely unclear, β-amyloid peptide (βAP) is thought to be a key role-player in both the brain and periphery. Here we studied the electro-genesis of skeletal muscle fibers in a mouse transgenic AD model. Membrane potential was recorded by standard electro-physiological techniques. Compared to wild-type rodents, AD mice show severe disturbances in skeletal muscle electro-genesis manifested by significant depolarization of myofibers. These changes are not affected by short-term βAP treatment, the mark of a chronic degenerative process in the periphery directly related to AD whereby ion pumps on muscle membranes exhibit reduced activity. This phenomenon may explain ionic imbalance and cellular dysfunction both in the neuro-muscular system and in the brain. The observed motor disturbances might play a key role in impaired activities of daily living, and addressing the muscular patho-physiology could improve quality of life in AD.

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1. Introduction

Alzheimer’s disease (AD) is a slowly progressive primary neurodegenerative disorder. Even though it is characterized mainly by memory impairment, piling evidence suggests that this is a systemic illness with the most prominent pathology in the cognitive functions of the central nervous system (CNS). Indeed, the neurotoxic β-amyloid peptide (βAP) which is thought to be one of the pathogenic factors, is ubiquitously found both in the brain and in the periphery, and its toxic effects were demonstrated in various tissues, including cellular blood (such as erythrocytes, lymphocytes), fibroblasts and muscles (Dolman, 1984; Eckert et al., 1998; Etcheberrigaray and Bhagavan, 1999; Kalmán et al., 1994; Li and Kaminakas, 1985; Mórocz et al., 2002; Mukhamedyarov et al., 2009, 2011; Palotás et al., 2002; Paoletti and Tombaccini, 1998; Peterson et al., 1985; Sevush et al., 1998; Soininen et al., 1992; Zubenko et al., 1984).

Research into non-cognitive symptoms is gaining ground as AD patients exhibit tremor, brady-kinesia, myoclonus, rigidity, gait and balance problems, apraxia, oculo-motor disturbances, etc. (Hebert et al., 2010; Scarmeas et al., 2005; Swanberg et al., 2004; vanHalteren-vanTilborg et al., 2007; Wilson et al., 2000; Wirths and Bayer, 2008). Given the shared symptoms in distinct neurodegenerative disorders, these motor dysfunctions are often difficult to interpret in cognitively impaired individuals. Sometimes patients with Parkinson’s disease (PD) may also develop dementia (PD–dementia complex), and AD not infrequently overlaps with PD (AD/PD) as well. Although in the majority of cases motor disturbances are seen in PD with dementia or in AD/PD, and can clinically be explained by advanced age, however a number of patients have motor symptoms directly related to AD, which might be attributable to βAP toxicity: indeed, levels of βAP are significantly increased in the skeletal muscles of AD patients (Kuo et al., 2000). Motor function is often compromised in individuals with mild cognitive impairment (MCI) as well, and such disturbance actually constitutes a risk factor for developing AD (Aggarwal et al., 2006; Buchman and Bennett, 2011). In addition, neuro-motor problems also indicate an increased risk of psychosis in AD patients (Caligiuri et al., 2003).

AD-specific muscle disturbances can affect both skeletal and cardio-myocytes (Leushina et al., 2012; Mukhamedyarov et al., 2009, 2011). We have previously shown that βAP disrupts resting...
membrane potential (RMP) of skeletal muscle fibers, leading to a pronounced depolarization. This is mainly due to the inhibition of Na\textsuperscript{+}/K\textsuperscript{+}-ATPase, as well as the formation of βAP-pores which subsequently increase membrane permeability (Mukhamedyarov et al., 2011, 2013). Moreover, βAP is known to impair muscle contractility in warm- and cold-blooded animals, however little is known about these effects in AD. The aim of this study, therefore, was to investigate the electro-genesis of skeletal muscle fibers in AD transgenic mice.

2. Methods

2.1. Preparation, solutions and chemicals

Experiments were carried out on the diaphragmatic muscle of B6C3-Tg(APP695)85Dbo Tg(PSEN1)85Dbo double transgenic (APP/PS1) mice expressing a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695sw) and a mutant human presenilin-1 (PS1-dE9), Both mutations are associated with early-onset AD (Mukhamedyarov and Zefirov, 2013). APP/PS1 rodents develop βAP deposits in brain and memory impairment by 6–8 months of age (Savonenko et al., 2005). APP/PS1 mice line was purchased from Jackson laboratory (USA) and bred at the Puschino animal facility branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry (Russian Academy of Science). APP/PS1 mice at the age of 3–7 months were delivered to Kazan State Medical University (Kazan, Russia), where rodents were housed under standard laboratory conditions, with a 12-h light/dark cycle and unlimited access to food and water. Experiments were performed on 3–4 and 7–8 months old APP/PS1 animals, as well as on age-matched wild-type (WT) mice as controls. The study protocol was approved by the local ethical committee of Kazan State Medical University.

Diaphragmatic muscle preparations were placed in experimental chambers with a perfusion solution containing NaCl (125 mM), KCl (2.5 mM), CaCl\textsubscript{2} (2 mM), Na\textsubscript{2}HPO\textsubscript{4} (1 mM), Mg\textsubscript{2}Cl\textsubscript{2} (1 mM), glucose (11 mM). The pH was maintained between 7.2–7.4 at 20 °C, and the solution was aerated for 1 h with carbogen (95% O\textsubscript{2}, 5% CO\textsubscript{2}) before the experiments. Na\textsuperscript{+}-free conditions were achieved by equimolarly substituting NaCl with Tris\textsuperscript{+}. Preparations with modified concentrations of extracellular K\textsuperscript{+} ([K\textsubscript{o}]\textsubscript{o}) maintained iso-osmolality by adjusting NaCl concentrations as appropriate. Where βAP was used, the 25–35 active fragment of the full-length neuro-toxic peptide (βAP\textsubscript{25-35}, EZBiolab Inc., USA) was added to the perfusion solution at a final concentration of 10\textsuperscript{-6} M, 2hrs prior to registering RMP. βAP\textsubscript{25-35} has the functional domain of full-length βAP required for both neuro-trophic and neuro-toxic effects (Kowall et al., 1992). The Na\textsuperscript{+}/K\textsuperscript{+}-ATPase-inhibitor ouabain (6 × 10\textsuperscript{-5} M) was added 30 min before monitoring RMP. All chemicals except βAP\textsubscript{25-35} were purchased from Sigma–Aldrich (USA).

2.2. Electro-physiology

Registration of RMP of muscle fibers was performed intra-cellularly under visual control by standard technique using glass micro-electrodes with 5–9 MΩ tip resistance, filled with 2.5 M KCl. RMP was assessed alternately in synaptic (i.e. in close proximity to the nerve ending) and extra-synaptic areas (i.e. few millimeters away from the nerve endings) of muscle fibers. In each case, RMP was measured in 25–30 parallel fibers. An additional criteria for finding of synaptic region of muscle was presence of miniature end-plate potentials.

Monitoring RMP was started 2 h after preparing the diaphragmatic muscles, as it was shown previously that RMP in synaptic, but not yet in extra-synaptic areas of muscle fibers is decreased 2 h after muscle preparation due to the development of primary denervation changes (i.e. inactivation of Na\textsuperscript{+}/K\textsuperscript{+}-ATPase) (Volkov, 1989; Volkov et al., 1985, 1987).

2.3. Statistical calculations

Statistical analysis was performed using MicrocalOrigin 7.5 program. All data are represented as mean ± standard error. The statistical significance of differences between samples was evaluated by Student’s t-test.

3. Results

3.1. Electro-genesis of skeletal muscle fibers in WT mice of different ages

RMP of skeletal muscle fibers in 3–4 months old WT mice at normal [K\textsubscript{o}]\textsubscript{o} (2.5 mM) was −78.2 ± 1.1 mV (n = 95) in the synaptic and −81.8 ± 1.2 mV (n = 96) in the extra-synaptic areas. This indicates a statistical difference, which remained significant (P < 0.05) at all [K\textsubscript{o}]\textsubscript{o} that were measured. The synaptic and extra-synaptic RMP-[K\textsubscript{o}]\textsubscript{o} curves were essentially parallel, and their slopes indicated a near-linear dependence on the intra- and extra-cellular [K\textsubscript{o}]\textsubscript{o} ratio (Fig. 1A). These characteristic differences between synaptic and extra-synaptic RMPs are known to be attributable to post-denervation changes in the electro-genic Na\textsuperscript{+}/K\textsuperscript{+}-ATPase following the loss of neuro-trophic control (Volkov et al., 1987). Synaptic and extra-synaptic muscular RMPs in 7–8-month-old WT mice at normal [K\textsubscript{o}]\textsubscript{o} were −83.2 ± 0.9 mV (n = 94) and −87.6 ± 0.8 mV (n = 98), respectively, and RMP dependence on [K\textsubscript{o}]\textsubscript{o} was similar to those seen in 3–4 months old rodents (Fig. 1B). These data demonstrate similar electro-genesis in younger and older mouse muscle fibers.

3.2. Impaired electro-genesis in skeletal muscle fibers of APP/PS1 mice

RMP of skeletal muscle fibers in 3–4 months old AD mice at normal [K\textsubscript{o}]\textsubscript{o} was −81.6 ± 0.9 mV (n = 97) in the synaptic, and −86.2 ± 0.7 mV (n = 96) in the extra-synaptic areas (Fig. 1C). These data are comparable with that seen in control animals and demonstrate intact electro-genesis in skeletal muscles of young APP/PS1 mice.

Significantly lower (P < 0.05) synaptic and extra-synaptic RMPs were seen in 7–8 months old AD mice at normal [K\textsubscript{o}]\textsubscript{o} (−67.8 ± 1.8 mV (n = 105) and −72.2 ± 1.7 mV (n = 100), respectively) when compared to either younger or older WT mice. Curves of RMP dependence on [K\textsubscript{o}]\textsubscript{o} was largely parallel in AD at [K\textsubscript{o}]\textsubscript{o} between 2.5–10 mM, similarly with that seen in controls. RMP values at [K\textsubscript{o}]\textsubscript{o} < 0.1 mM, however, markedly shifted to less negative numbers, and at [K\textsubscript{o}]\textsubscript{o} > 10 mM the curves started to converge (Fig. 1D), the mark of a significant impairment of skeletal muscle electrogensis in older AD mice.

3.3. Identifying mechanisms of impaired electro-genesis in APP/PS1 mouse muscle fibers

In order to identify the possible contribution of changes in membrane Na\textsuperscript{+} permeability to depolarization of muscle fibers in APP/PS1 mice, experiments with Na\textsuperscript{+}-free solutions were conducted. Under these conditions, depolarization of muscle fibers was even more pronounced: RMP at normal [K\textsubscript{o}]\textsubscript{o} in synaptic regions was −58.3 ± 0.9 mV (n = 105), whereas the differences in RMP values between synaptic and extra-synaptic areas of muscle fibers disappeared (Fig. 2A). RMPs in AD mice were less negative in a Na\textsuperscript{+}-free climate when compared to readings recorded under normal Na\textsuperscript{+} concentrations at various [K\textsubscript{o}]\textsubscript{o} (P < 0.05) except for...
Similar changes were found following the application of the Na\(^{+}/K\)-ATPase-blocker ouabain, however changes in depolarization of muscle fibers were more prominent than in the Na\(^{+}\)-free medium (Fig. 2B). Treatment with ouabain resulted in less negative RMP values when compared to control preparations at all tested [K\(^{+}\)]\(_{o}\) (P < 0.05). These suggest that Na\(^{+}/K\)-ATPase is not severely affected in AD, and no changes in Na\(^{+}\)-permeability are present in muscle fibers of APP/PS1 mice that might contribute to the altered depolarization.

3.4. Effects of \(\beta\)AP on the electro-genesis in muscle fibers of AD mice

We have previously found that \(\beta\)AP elicits pronounced depolarization of skeletal muscle fibers in WT mice by inhibiting Na\(^{+}/K\)-ATPase activity and increasing cationic membrane permeability by forming amyloid-pores (Mukhamedyarov et al., 2013). Impact of \(\beta\)AP on RMP, therefore, was evaluated in APP/PS1 mice. \(\beta\)AP\(_{25-35}\) (10\(^{-6}\) M) caused depolarization in muscle fibers at normal [K\(^{+}\)]\(_{o}\): the value of −60.5 ± 1.2 mV (n = 97), however, is less pronounced when compared to that seen in WT mice (Mukhamedyarov et al., 2011, 2013). With the exception of [K\(^{+}\)]\(_{o}\) = 0.1 and 10 mM, \(\beta\)AP\(_{25-35}\) shifts RMP to less negative values irrespective of [K\(^{+}\)]\(_{o}\) (P < 0.05). The neuro-toxic \(\beta\)AP also diminished the differences between RMPs in synaptic and extra-synaptic areas (Fig. 2C). These effects confirm the blocking of Na\(^{+}/K\)-ATPase by \(\beta\)AP, which is broadly in line with previous findings (Mukhamedyarov and Zefirov, 2013). However, the less pronounced depolarization of muscle fibers and preservation of RMP slope dependence on [K\(^{+}\)]\(_{o}\) suggest that \(\beta\)AP apparently does not increase cationic permeability of AD muscle fibers, only in WT mice (Mukhamedyarov et al., 2013).

4. Discussion

Although AD is characterized mainly by dementia, non-cognitive symptoms such as motor dysfunction are also of clinical significance, but the patho-mechanism is largely unknown. Here we show that RMP generation in AD mice is severely disrupted: muscle fibers are depolarized by approximately 15 mV. The neuro-toxic \(\beta\)AP is known to form non-selective amyloid pores (Mukhamedyarov et al., 2013) that might explain these changes, however APP/PS1 mice showed neither increased Na\(^{+}\) membrane permeability, nor significantly impaired Na\(^{+}/K\)-ATPase activity. Nevertheless, a slight undetected functional change in the ion pumps, such as in Na\(^{+}/K\)-ATPase, might lead to decreased transmembrane K\(^{+}\) concentration gradient and therefore a decline in RMP. Ion-pumps can be rendered sluggish in AD as result of the chronic toxic effect of \(\beta\)AP causing sustained low-grade oxidative stress, Ca\(^{2+}\)-dyshomeostasis, subtle changes in lipid composition of the cell membrane, up/down-regulation of various intra-cellular signaling cascades, etc. (Kourie, 1998, 2001; Mukhamedyarov and Zefirov, 2013).

Even though \(\beta\)AP effectively erased the differences in RMP between synaptic and extra-synaptic areas, suggesting blockade on Na\(^{+}/K\)-ATPase, muscle fibers of APP/PS1 mice appear to be less sensitive to the effects of \(\beta\)AP than what was published earlier in control rodents (Mukhamedyarov et al., 2013). Specifically, \(\beta\)AP yields less marked depolarization in AD, indicating a degree of resistance to this peptide, which might be attributable to a number of factors. Short-term application of \(\beta\)AP on muscle fibers of control animals can be seen as an acute impact, however AD pathology develops during the entire life of APP/PS1 mice, reflecting a chronic effect of this neuro-toxic peptide. In addition, the double transgenic AD strain used in this study carries mutant genes of both APP and PS1, the latter of which having numerous effects not related to \(\beta\)AP metabolism (Honarnejad and Herms, 2012). AD mice express a number of various species of \(\beta\)AP with different length and characteristics, whereas \(\beta\)AP models expose muscle fibers to only a single short-length form and at a concentration not accurately reflecting \(\beta\)AP levels in the muscular system of APP/PS1 animals. Long-term adaptive mechanisms might also exist in AD mice, such as immune responses and increased activity of \(\beta\)AP-degrading enzymes (Mukhamedyarov and Zefirov, 2013), which are virtually absent in isolated muscle preparations evaluating \(\beta\)AP in vitro.
characterized, especially in the advanced stages, by mild-to-moderate motor dysfunction (Scarpace et al., 2005; Wilson et al., 2000: Wirths and Bayer, 2008), and the observed changes in RMP might, at least in part, explain these signs and symptoms. Demented patients often show weight loss as a result of a decrease in muscle mass, reflecting the degenerative process in the motor system (Poehlman and Dvorak, 2000). In the majority of cases, however, the neuro-muscular impairment is neither flagged up, nor diagnosed perhaps because the progressive decline is generally put down to poor mental status and deterioration of general condition. Patients with severe dementia gradually become bed-ridden, however the underlying muscular pathology as a real cause might never surface. Activities of daily living (ADL) are affected in AD, and its decline is one of the diagnostic criteria. Although cognitive dysfunction is thought to lead to impaired day-to-day functioning, it is reasonable to hypothesize that the interference with ADL is actually attributable, at least in part, to AD-specific muscular dysfunction. Disturbed electro-genesis of muscle fibers negatively affect physiological processes in skeletal muscles, including neuro-muscular transmission, generation of action potential, myo-fiber contraction, various regulatory mechanisms, etc., all of which can explain the muscular pathology seen in AD. Further characterizing and clinically addressing non-cognitive symptoms such as motor impairment, therefore, might improve ADL in this devastating disorder.

Conflict of interest

The authors declare no conflict of interest.

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Fig. 2. Depolarization of skeletal muscle fibers in 7–8 months old APP/PS1 mice under different conditions. Association between MP and [K+]o in the synaptic (black squares) and extra-synaptic (white circles) zones of muscle fibers is depicted in Na+-free solution (A), under the action of 6 × 10⁻⁵ M of ouabain (B), and following treatment with 10⁻⁶ M of βAP (25-35) (C).

Young (3–4 months old) APP/PS1 rodents do not yet suffer from accumulation of βAP, and only develop amyloid-deposits in the brain with subsequent memory impairment by 6–8 months of age (Savonenko et al., 2005). As RMP disturbances were found only in 7–8 month old mice with clinically manifested AD, but not in younger animals in our study, disrupted muscular electro-genesis in AD mice appears to be directly related to the patho-physiology of the dementing illness. As such, this report also provides evidence that the alterations of muscle membrane potential are not due to the transgenes of APP/PS1 per se, but to the AD itself where-by the disease itself generates a (possibly secondary) muscular-phenomenon.

Disturbed electro-genesis in muscle fibers has important clinical relevance. Apart from memory impairment, AD is also


