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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.

1. Introduction

Alzheimer's disease (AD) is a slowly progressive primary neuro-degenerative 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 neuro-toxic β-amyloid peptide (βAP) which is thought to be one of the pathognomic 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; Kálmán et al., 1994; Li and Kaminskas, 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 neuro-degenerative 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

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membrane potential (RMP) of skeletal muscle fibers, leading to a pronounced depolarization. This is mainly due to the inhibition of Na^+/K^+-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/HuAPP695swe) 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), $CaCl_2$ (2 mM), NaH_2PO_4 (1 mM), $MgCl_2$ (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_2, 5% CO_2) before the experiments. Na^+-free conditions were achieved by equimolarly substituting NaCl with $Tris^+$. Preparations with modified concentrations of extracellular K^+($[K^+]_o$) maintained iso-osmolarity by adjusting NaCl concentrations as appropriate. Where βAP was used, the 25–35 active fragment of the full-length neuro-toxic peptide ($βAP_{25-35}$, EZBiolab Inc., USA) was added to the perfusion solution at a final concentration of 10–6 M, 2hrs prior to registering RMP. $βAP_{25-35}$ has the functional domain of full-length βAP required for both neuro-trophic and neuro-toxic effects (Kowall et al., 1992) The Na^+/K^+ATPase-inhibitor ouabain (6×10^{-5} M) was added 30 min before monitoring RMP. All chemicals except $βAP_{25-35}$ 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 microelectrodes 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^+/K^+-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 $[K^+]_o$ (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 $[K^+]_o$ that were measured. The synaptic and extra-synaptic RMP-$[K^+]_o$ curves were essentially parallel, and their slopes indicated a near-linear dependence on the intra- and extra-cellular $[K^+]$ 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^+/K^+-ATPase following the loss of neuro-trophic control (Volkov et al., 1987). Synaptic and extra-synaptic muscular RMPs in7–8-month-old WT mice at normal $[K^+]_o$ were −83.2 ± 0.9 mV ($n = 94$) and −87.6 ± 0.8 mV ($n = 98$), respectively, and RMP dependence on $[K^+]_o$ 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 $[K^+]_o$ 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 $[K^+]_o$ (−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 $[K^+]_o$ was largely parallel in AD at $[K^+]_o$ between 2.5–10 mM, similarly with that seen in controls. RMP values at $[K^+]_o \leqslant 0.1$ mM, however, markedly shifted to less negative numbers, and at $[K^+]_o \geqslant 10$ mM the curves started to converge (Fig. 1D), the mark of a significant impairment of skeletal muscle electro-genesis 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^+ permeability to depolarization of muscle fibers in APP/PS1 mice, experiments with Na^+-free solutions were conducted. Under these conditions, depolarization of muscle fibers was even more pronounced: RMP at normal $[K^+]_o$ 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^+-free climate when compared to readings recorded under normal Na^+concentrations at various $[K^+]_o$ ($P < 0.05$) except for $[K^+]_o = 0.1$ and 10 mM. Similar changes were found following the

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