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The increased potassium intake improves cognitive performance and attenuates histopathological markers in a model of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by hallmarks that include an accumulation of amyloid-β peptide (Aβ), inflammation, oxidative stress and synaptic dysfunction, which lead to a decrease in cognitive function. To date, the onset and progression of AD have been associated with pathologies such as hypertension and diabetes. Hypertension, a disease with a high incidence worldwide, is characterized by a chronic increase in blood pressure. Interestingly, this disease has a close relationship to the eating behavior of patients because high Na^+ intake is a significant risk factor for hypertension. In fact, a decrease in Na^+ consumption, along with an increase in K^+ intake, is a primary non-pharmacological approach to preventing hypertension. In the present work, we examined whether an increase in K^+ intake affects the expression of certain neuropathological markers or the cognitive performance of a murine model of AD. We observed that an increase in K^+ intake leads to a change in the aggregation pattern of the Aβ peptide, a partial decrease in some epitopes of tau phosphorylation and improvement in the cognitive performance. The recovery in cognitive performance was correlated with a significant improvement in the generation of long-term potentiation. We also observed a decrease in markers related to inflammation and oxidative stress such as glial fibrillary acidic protein (GFAP), interleukin 6 (IL-6) and 4-hydroxynonenal (4-HNE). Together, our data support the idea that changes in diet, such as an increase in K^+ intake, may be important in the prevention of AD onset as a non-pharmacological therapy.

1. Introduction

Alzheimer's disease is the most common cause of cognitive impairment. This neurological disorder is characterized by the presence of neurofibrillary tangles, senile plaques (composed by the amyloid-β) and synaptic dysfunction. Other features of AD include oxidative stress, inflammation, Ca^{+2} imbalance, and disruption of signaling pathways [1–3]. In recent years, several clinical studies have proposed what may be an important factor in the progression of AD [4,5]. In fact, calorie restriction, the Mediterranean diet and drinking red wine, rich in polyphenols have been reported to attenuate AD deterioration of spatial memory, delay the onset of dementia and reduce amyloid neuropathology [5–7]. Epidemiological studies also suggest a relationship between AD and systemic pathologies such as metabolic syndrome, obesity and type II diabetes [5]. A common factor in these AD-related pathologies is a chronic increase in blood pressure. Hypertension is defined as persistent systolic blood pressure ≥ 140 mm Hg and diastolic blood pressure ≤ 90 mm Hg. Overall, approximately 20% of the population worldwide has hypertension, and its incidence is increasing [8]. Hypertension is considered a risk factor for several diseases, including hemorrhagic and ischemic stroke [9]. Furthermore, several studies have reported that blood pressure is increased in AD patients, even decades before the onset of the disease [5]. Furthermore, hypertension during midlife has been associated with an increase in the number of senile plaques in the neocortex and hippocampus and an augmentation of neurofibrillary tangles in the hippocampus. Therefore, prevention of hypertension may attenuate the onset or progression of AD [5,10–12].

It has been reported that increase in K^+ intake is beneficial for blood pressure and has no adverse effects [13–18]. In addition, several antihypertensive agents have shown some prevention of cognitive decline, diminishing the appearance of dementia related to AD in approximately 50% of study subjects [19]. In other reports, diuretics specifically, potassium-sparing diuretics to treat high blood pressure had an effect on dementia. These diuretics are associated with an important reduction of AD risk, and it is the antihypertensive treatment that has the

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greatest effect [20]. To study the effect of hypertension prevention on the onset and progression of AD, we studied the effect of a diet enriched in K^+. In a typical recommended diet, dietary intake of K^+ is at least 120 mmol/day (≈4.7 g/day); however, in the Western diet, Na^+ consumption is high and K^+ consumption is less than half of the recommended amount [14,21,22]. This low K^+ consumption has been associated with diseases such as hypertension, stroke and cardiovascular diseases [13,21,23].

In the present work, we used an animal model of AD: 4-month-old transgenic APPswe/PSEN1 mice. The mice received 2% potassium in their water for 8 weeks. Four-month-old animals were chosen because at that age neither significant cognitive loss nor brain Aβ plaques are detectable compared with 6-month-old mice (age at the end of treatment), which exhibit high brain Aβ content and memory loss [24–26]. We observed that treatment with 2% potassium significantly improved cognitive performance, mainly in learning and memory, suggesting a recovery in hippocampal activity. The improvement in cognitive performance was correlated with an increase in LTP generation and a decrease in inflammatory markers such us IL-6 and GFAP and the oxidative stress marker 4-HNE. Together, these results suggest that an increase in K^+ intake results in the prevention of cognitive alterations and oxidative stress in the early stages of AD in a mouse model.

2. Methods

2.1. Animals and treatment

Male APPswe/PS1dE9 (4-month-old) and C57BL/6 (6-month-old) mice were used in this study. APP/PS1 animals co-express a Swedish (K594M/N595L) mutation of a chimeric mouse/human APP (Mo/HuAPP695swe) together with the human exon-9-deleted variant of PS1 (PS1-dE9); these mice secrete elevated amounts of human Aβ peptide. This strain was obtained from The Jackson Laboratory (Bar Harbor, ME, USA). The animals (wild type and APP) were separated into two groups (17 animals by group); one group served as the control, the other group was supplemented with 2% potassium chloride in the drinking water [27]. The duration of the treatment was 8 weeks, including the behavioral tests. Mice were fed ad libitum. They were housed at the Animal House Facility of the Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. After treatment the mice were used in the following order: 9 animals for the cognitive test and blood analysis, 5 animals for electrophysiology experiments and 3 animals for immunoblotting and immunofluorescence analysis.

2.2. Biochemical analysis

Blood was collected from the tail vein after 6 h of fasting and then centrifuged at 2500 × *g* for 10 min at room temperature to obtain serum samples. Glucose levels were measured using the hexoquinase/G-6-PDH method in the Architect Analyzer (Abbott Laboratories, Abbott Park, IL, USA), and insulin levels were measured via chemiluminescence (Beckman Coulter); in both cases, the manufacturers' instructions were followed. Triglyceride and cholesterol were assayed enzymatically in the Architect c8000 (Abbott Laboratories, USA).

2.3. Thioflavin-S (Th-S) staining

To detect amyloid formation, Th-S staining was developed using brain slices mounted on gelatin-coated slides as previously described [28,29]. Slices were dehydrated and rehydrated in xylene and ethanol baths, followed by incubation in distilled water for 10 min. Next, slices were immersed in Th-S solution (0.1% ThS in 70% ethanol) for 5 min and then washed twice in 70% ethanol for 30 s and cover-slipped with mounting medium in the dark.

2.4. ELISA of $A\beta_{42}$ and $A\beta_{40}$ peptides

The brain samples were extracted on ice and immediately processed as previously described [30]. Briefly, tissues were homogenized in RIPA buffer (10 mM Tris–Cl, pH 7.4, EDTA 5 mM, 1% NP-40, 1% sodium deoxycholate, and 1% SDS) supplemented with a protease inhibitor mixture (1 mM PMSF, 2 μg/mL aprotinin, 1 μg/mL pepstatin and 10 μg/mL benzamidine) and phosphatase inhibitors (25 mM NaF, 100 mM Na_3VO_4, 1 mM EDTA and 30 μM $Na_4P_2O_7$) using a Potter homogenizer and then passed sequentially through different caliber syringes. Protein samples were centrifuged at 14,000 rpm at 4 °C twice for 15 min. Then, brain Aβ levels from 50 μL of the processed samples were determined using Human Amyloid β_{40} and Amyloid β_{42} Brain ELISA (Millipore), and the absorbance of each plate was read in a spectrophotometer (Metertech).

2.5. Immunofluorescence

Immunofluorescence in brain slices was performed as described previously [31,32]. Slices were washed three times in ice-cold PBS and then permeabilized for 30 min with 0.2% Triton X-100 in PBS. After several rinses in ice-cold PBS, the samples were incubated in blocking solution (0.2% bovine serum albumin in PBS) for 1 h at room temperature followed by an overnight incubation at 4 °C with primary antibodies. After primary antibody incubation, the slices were extensively washed with PBS and then incubated with Alexa-conjugated secondary antibodies (Molecular Probes, Carlsbad, USA) for 2 h at 37 °C. The primary antibodies used were rabbit 4G8 and mouse anti-6E10 (Covance, Princeton, USA), rabbit anti-IL-6 (Abcam, Cambridge, UK), rabbit nitrotyrosine (Abcam, Cambridge, UK), rabbit 4-HNE (Abcam, Cambridge, UK), and rabbit anti-GFAP (Dako, Denmark). The nuclear stain was performed by treating the slices with Hoechst (Sigma-Aldrich, St. Louis, USA). The slices were subsequently mounted on slides using mounting medium and analyzed using a Zeiss LSM 5 Pascal confocal microscope. The images were analyzed using NIH Image J software.

2.6. Tricine-SDS PAGE

The brain samples were extracted on ice and immediately processed as previously detailed [33]. Briefly, tissues were homogenized in RIPA buffer (10 mM Tris–Cl, pH 7.4, EDTA 5 mM, 1% NP-40, 1% sodium deoxycholate, and 1% SDS) supplemented with a protease inhibitor mixture (1 mM PMSF, 2 μg/mL aprotinin, 1 μg/mL pepstatin and 10 μg/mL benzamidine) and phosphatase inhibitors (25 mM NaF, 100 mM Na_3VO_4, 1 mM EDTA and 30 μM $Na_4P_20_7$) using a Potter homogenizer and then passed sequentially through different caliber syringes. Protein samples were centrifuged at 14,000 rpm at 4 °C twice for 15 min. Then, the proteins (100 μg) were separated by electrophoresis performed in a Tris–Tricine buffer system [0.2 M Tris (pH 8.9) as an anode buffer and 0.1 M Tris, 0.1 M Tricine, 0.1% SDS (pH 8.25) as a cathode buffer] and then transferred to a PVDF membrane. The transfers were followed by incubation with the primary antibody 4G8 (Covance) and anti-mouse IgG peroxidase conjugated antibody (Pierce, USA) and developed using an ECL kit (Western Lightning Plus ECL, PerkinElmer).

2.7. Immunoblotting

The hippocampus was removed from each brain and homogenized in RIPA buffer (50 mM, Tris–Cl, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 1% SDS) supplemented with a protease inhibitor cocktail (Sigma-Aldrich P8340) and phosphatase inhibitors (50 mM NaF, 1 mM Na_3VO_4 and 30 μM $Na_4P_20_7$) using a Potter homogenizer and then passed sequentially through different caliber syringes. Protein samples were centrifuged at 14,000 rpm at 4 °C twice for 15 min. Protein concentration was determined using the Bicinchoninic Acid (BCA) Protein Assay Kit (Pierce Biotechnology, Rockford, USA). Samples of