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Quantitative Measurement of [Na+] and [K+] in Postmortem Human Brain Tissue Indicates Disturbances in Subjects with Alzheimer's Disease and Dementia with Lewy Bodies

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Abstract. Alzheimer's disease (AD) is associated with significant disturbances in the homeostasis of Na+ and K+ ions as well as reduced levels of Na+/K+ ATPase in the brain. This study used ICP-MS to accurately quantify Na+ and K+ concentrations in human postmortem brain tissue. We analyzed parietal cortex (Brodmann area 7) from 28 cognitively normal age-matched controls, 15 cases of moderate AD, 30 severe AD, and 15 dementia with Lewy bodies (DLB). Associations were investigated between [Na+] and [K+] and a number of variables including diagnosis, age, gender, Braak tangle stage, amyloid-β (Aβ) plaque load, tau load, frontal tissue pH, and *APOE* genotype. Brains from patients with severe AD had significantly higher (26%; $p < 0.001$) [Na+] (mean $65.43 \pm$ standard error 2.91 mmol/kg) than controls, but the concentration was not significantly altered in moderate AD or DLB. [Na+] correlated positively with Braak stage ($r = 0.45$; $p < 0.0001$), indicating association with disease severity. [K+] in tissue was 10% lower ($p < 0.05$) in moderate AD than controls. However, [K+] in severe AD and DLB (40.97 ± 1.31 mmol/kg) was not significantly different from controls. There was a significant positive correlation between [K+] and Aβ plaque load ($r = 0.46$; $p = 0.035$), and frontal tissue pH ($r = 0.35$; $p = 0.008$). [Na+] was not associated with [K+] across the groups, and neither ion was associated with tau load or *APOE* genotype. We have demonstrated disturbances of both [Na+] and [K+] in relation to the severity of AD and markers of AD pathology, although it is possible that these relate to late-stage secondary manifestations of the disease pathology.

Keywords: Alzheimer's disease, dementia with Lewy bodies, human brain, ICP-MS, potassium, sodium

INTRODUCTION

Alzheimer's disease (AD) is a complex neurodegenerative disorder for which there are no disease modifying therapies [1]. The pathological characteristics of AD include the accumulation of extracellular Aβ plaques and the formation of intraneuronal

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neurofibrillary tangles composed of hyperphosphorylated microtubule-associated tau protein [2, 3]. The biochemical processes that initiate AD remain unknown, but it is clear that multiple pathways are involved in the development of the pathological characteristics of the disease.

Ion pools of Na+ and K+ are critical for nerve signal transduction, electrophysiological activity, membrane transport, and other cellular processes. It has been speculated that imbalance of these ions occurs in brain astrocytes in AD, possibly secondary to the accumulation of Aβ [4]. However, the evidence that there are significant Na+/K+ perturbations in AD brain is somewhat limited. It has been well-documented that AD is associated with depressed ATPase activity in the brain [5], and increased Na+ dependent Ca2+ uptake has been reported in AD brain tissue [6]. The key limitation in almost all studies is that they use indirect methods to measure changes these monovalent ions. A further limitation is that studies examining human brain tissue commonly use samples sizes that make it impossible to probe associations between K+ and Na+ and disease pathology. Significant and definitively quantifiable changes in brain Na+ and K+ ion homeostasis in human AD are a relatively recent finding [4]. Vitvitsky et al. [4] measured [Na+] and [K+] in 12 age-matched controls and 16 AD brains. They found that in AD samples [Na+] in the frontal and parietal cortex increased 20–25%, and in the cerebellum [K+] increased 15% [4]. It was suggested that these ion imbalances were largely due to changes in the intracellular compartment, because similar changes were not evident in cerebrospinal fluid (CSF) from AD patients. Aβ may contribute to this since exposure of astrocytes to Aβ *in vitro* increased intracellular levels of both Na+ and K+, possibly due to reduced gene expression of Na+/K+ ATPase and Na+-dependent glutamate transporters [4]. This basis for this is that the uptake of glutamate activates Na+/K+ ATPase (which acts to normalize the resting transmembrane gradients of Na+ and K+), but in AD brain the levels and functioning of glutamate transporters appear to be reduced/impaired [7]. It may be relevant that oxidative modification and impaired functioning of the glial glutamate transporter was reported in synaptosomes treated with Aβ [7]. Resting transmembrane ion gradients could be further impacted by the extent to which Na+/K+ ATPase is reduced in the brain of AD subjects [5]. We wished to explore whether disturbances in brain [Na+] and [K+] in AD subjects were associated with key demographic variables, the stage of disease and the severity of pathological abnormalities.

In the present study, we measured [Na+] and [K+] in postmortem human brain tissue from patients with moderate or severe AD, dementia with Lewy bodies (DLB), and age- and gender-matched controls and explored the associations with age at death, gender, *APOE* genotype, Braak tangle stage, Aβ plaque load, tau load, and postmortem tissue pH.

MATERIALS AND METHODS

Human postmortem tissue

As described by Graham et al. postmortem tissue samples (parietal neocortex, Brodmann area 7) were obtained from pathologically and clinically confirmed cases of AD ($n = 45$), DLB ($n = 15$), and normal age-matched controls ($n = 28$) [8]. The AD was classified as moderate ('intermediate' AD neuropathological change [9], Braak tangle stage III–IV; $n = 15$) or severe ('high' AD neuropathological change [8], Braak tangle stage V–VI; $n = 30$). For comparison purposes, the control group also included tissue from two significantly younger individuals. Cases were geographically spread across the UK (Bristol, Newcastle, and London) and were obtained through the Brains for Dementia Research (BDR; see acknowledgments). The neuropathological diagnoses were made using widely accepted criteria [9, 10], uniformly applied according to a standardized protocol by members of the BDR Neuropathology Group. Consent and ethical approval for the use of tissue were obtained by individual brain banks all of which are licensed by the Human Tissue Authority (UK).

ICP-MS analysis

Frozen tissue samples (∼5 g) were lyophilized, milled to a fine powder, and 100 mg (±0.5 mg) was added to 2 ml of 69% Arsistar nitric acid in a sterile 15 ml centrifuge tube (Falcon). The mixture was allowed to sit overnight at room temperature and then 2 ml of 30% Suprapur hydrogen peroxide was added and degassed in a fume hood for 90 min. Samples were then heated in a microwave [CEM Mars 6 Microwave digester, Matthew NC, USA] (slow ramp to 55°C held for 5 min, ramped to 75°C held for 5 min, and finally ramped to 95°C and held for 30 min) and allowed to cool for 60 min. An internal standard (rhodium 10 μg/L) was included in all samples, standards, and blanks to correct for signal drift throughout the run. Samples, blanks, and trace element standards (0.1–100 μg/L) were analyzed, by inductively coupled

plasma–mass spectrometry (ICP-MS) [Thermo Scientific iCAP Q, Waltham MA, USA], using Helium gas at 4.5 ml/min in a reaction/collision cell to remove interferences. Certified reference material (CRM) NIST 1577b (Bovine liver) and European reference material (ERM) BB184 (Bovine muscle) were used with certified values of 1,800 mg/Kg (Na+) and 15,800 mg/Kg (K+) [8]. CRM recovery for Na+ was in the range 122–138% and 111–129% for K+. Each individual sample, standards, and blanks were corrected relative to the internal standard intensities and then this corrected internal standard signal intensity was used to compensate individually for any possible variation in overall signal intensity throughout the analytical run. Na+ or K+ concentrations were calculated by interpolation against calibration curves, first as mg/kg of dry tissue weight and then expressed as mmol/kg.

Tissue measurements of Aβ and tau

Aβ plaque load and tau load were determined as previously reported [11–13]. Frontal, temporal, and parietal lobe sections were labeled with anti-human Beta-Amyloid (clone 6F/3D) mouse monoclonal antibody from Dako (M0872) for analysis of parenchymal Aβ load. Aβ plaque load was calculated using Histometrix software, driving a Leica microscope with a motorized stage, as the percentage area of cerebral cortex immunopositive for Aβ after manual editing for exclusion of Aβ-laden vessels. After low-magnification selection of the area to be analyzed by the user and high-magnification calibration of the threshold labelling density, the software is programmed to select a defined number of regions (typically 30) at random within the selected area and to measure at high magnification the cumulative area fraction with a density exceeding the threshold value. A mean value was determined from the frontal, temporal, and parietal lobe measurements to give the total parenchymal Aβ plaque load. The tau load was ascertained in a similar manner in sections labeled with Anti-tau AT8 monoclonal mouse antibody from Autogen Bioclear (BR03).

Statistical analysis

Normality of distribution was assessed using the Anderson-Darling test. Na+ and K+ concentrations were found to be non-normally distributed. One-way Kruskal-Wallis test was used for comparisons between groups. Spearman's rank correlation coefficient was determined for correlation analysis.

RESULTS

Levels of sodium and potassium in brain tissue

The mean (±SEM) [Na+] in tissue from controls was 51.91 ± 1.47 mmol/kg (dry weight; Fig. 1A). In severe AD, [Na+] was significantly higher than in controls (65.43 ± 2.91 mmol/kg; $p < 0.001$), by approximately 26%. [Na+] in the moderate AD (55.90 ± 3.26 mmol/kg) and DLB (56.61 ± 2.04 mmol/kg) groups was not significantly different from that in controls.

The mean (±SEM) [K+] in tissue from controls was 40.58 ± 0.69 mmol/kg (dry weight; Fig. 1B). Cortex from patients with moderate AD had modestly lower [K+] (36.64 ± 1.26 mmol/kg) than either control (9.7%) or DLB brains (10.6%). However, [K+] in severe AD (37.85 ± 0.82 mmol/kg) and DLB (40.97 ± 1.31 mmol/kg) was not significantly different from control values.

There was no association between [Na+] or [K+] concentrations and gender. The correlation of age at death with [Na+] (Fig. 1C) and [K+] (Fig. 1D) was examined and there were no age-associations in any of the groups with one exception: a significant correlation between age at death and [K+] ($r = -0.54$; $p < 0.05$) in DLB. The relationship between [Na+] and [K+] was also examined and only in the control group was there a significant correlation ($r = 0.40$; $p = 0.03$; Fig. 1E).

Correlations of sodium and potassium levels with other pathological variables

Figure 2 shows the correlations of [Na+] and [K+] with Braak stage, Aβ plaque load, tau load, frozen tissue pH, and *APOE* genotype. Statistically significant positive correlations were observed between Na+ and Braak stage ($r = 0.45$; $p < 0.0001$; Fig. 2A); K+ and Aβ load ($r = 0.46$; $p = 0.0347$; Fig. 2D), and K+ and frontal tissue pH ($r = 0.35$; $p = 0.0081$; Fig. 2H). Neither [Na+] nor [K+] correlated with tau load or varied with *APOE* genotype.

DISCUSSION

This study measured Na+ and K+ concentrations in parietal cortex (Brodmann area 7) from 88 brains including 58 from patients with AD or DLB. Some interesting and potentially important associations between changes in brain [Na+] and [K+] and AD and DLB were apparent. [Na+] correlated with Braak tangle stage and [K+] with Aβ plaque load.

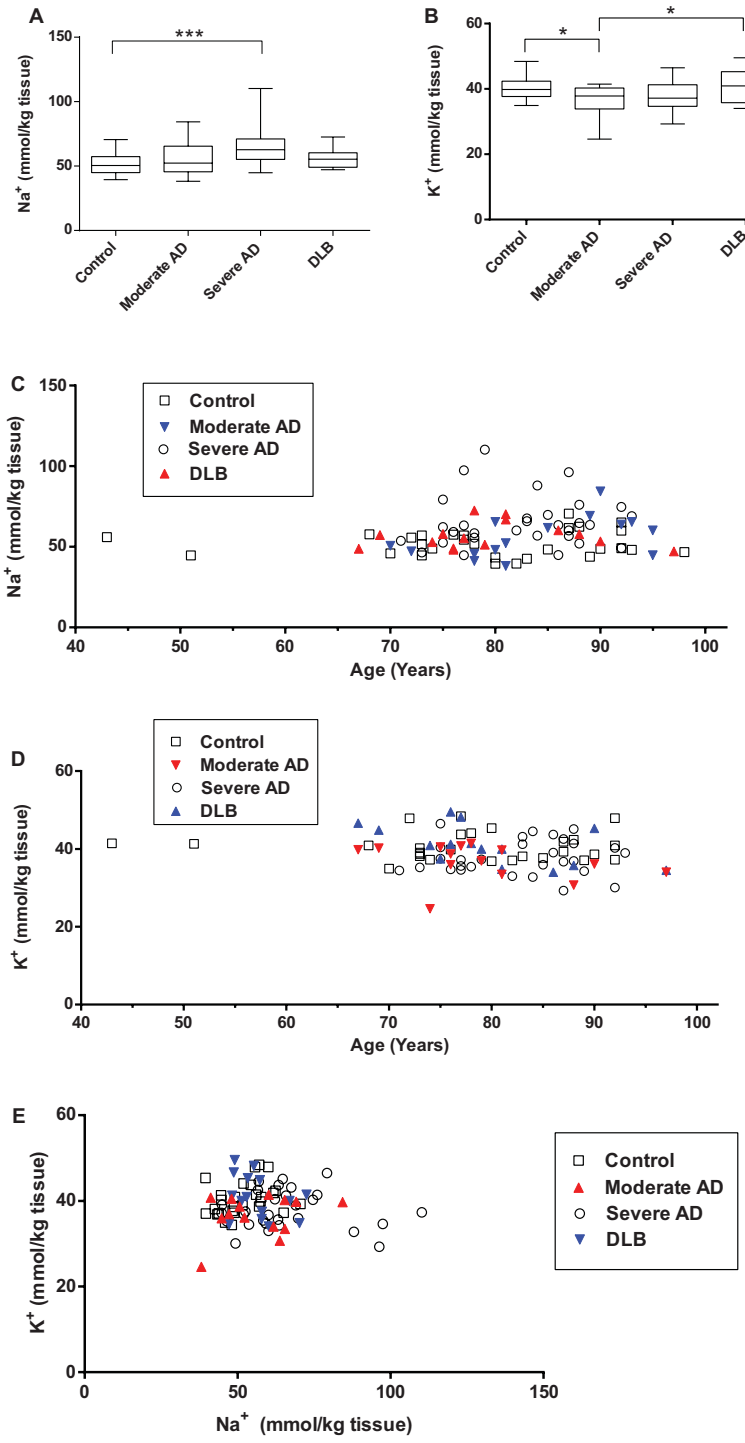

Fig. 1. Brain Na+ and K+ concentrations in AD and DLB subjects. (A) and (B) are box and whisker plots showing absolute levels of brain sodium and potassium (mmol/kg dry weight), respectively in healthy age-matched control subjects (Control; $n = 28$), moderate AD (Braak stages 3–4; $n = 15$), severe AD (Braak stages 5–6; $n = 30$) and DLB (dementia with Lewy bodies; $n = 15$). Statistical differences between groups (one way analysis of variance) are indicated. (C) and (D) illustrate brain sodium and potassium levels, respectively across the age distribution. The only significant correlation observed was between subject age and K+ concentrations in the DLB group (Spearman $r = -0.54$; $p > 0.05$). (E) The relationship between Na+ concentrations and K+ concentrations was examined. The only subject group with a significant correlation was the control group (Spearman $r = 0.40$; $p < 0.05$).

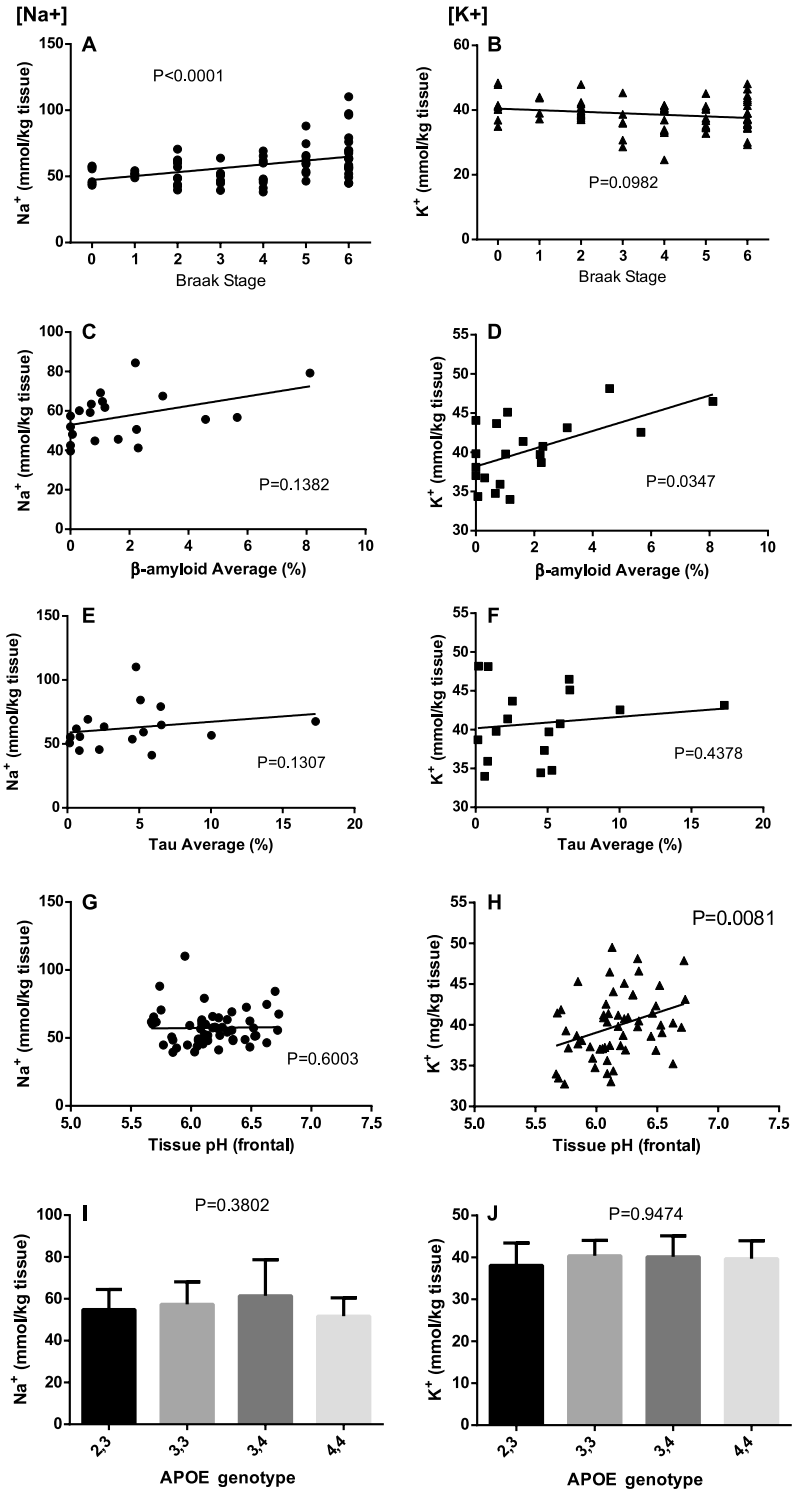

Fig. 2. Correlation of brain Na+ and K+ concentrations against other parameters. The relationship of Na+ (LHS) and K+ (RHS) concentrations (mmol/kg) were plotted against Braak stage (A,B; $n = 88$), Aβ (C,D; $n = 32$), tau protein (E,F; $n = 17$), tissue pH (frontal) (G,H; $n = 56$), and *APOE* genotype (I,J; $n = 51$). Statistically significant correlations were observed between Na+ and Braak stage (Spearman $r = 0.45$; $p < 0.0001$), K+ and Aβ (Spearman $r = 0.46$; $p = 0.0347$), and K+ and frontal tissue pH (Spearman $r = 0.35$; $p = 0.008$). No significant correlations of Na+ or K+ against either tau protein or *APOE* genotype were found.

[Na+] was 26% higher in brain tissue from patients with severe AD than controls but was not elevated in moderate AD. This suggests that changes to [Na+] are a late-stage development, in keeping with the significant positive correlation between [Na+] and Braak stage. The increase in [Na+] seems not simply to be a consequence of late-stage neurodegeneration, since it was not found in DLB. Exposure to Aβ was shown to increase intracellular Na+ in a concentration-dependent manner in isolated murine astrocytes [4]. We did not find any correlation between [Na+] and the either Aβ or tau load. However, in this study we measured only Aβ that was immunohistochemically detectable in paraffin sections and cannot exclude an association with the various soluble forms of this peptide. There was no association between [Na+] and tissue pH or *APOE* genotype.

The concentration of K+ in brain tissue appeared to vary independently from that of Na+. [K+] was significantly lower in moderate AD than in controls or DLB, yet not significantly reduced in severe AD. Not surprisingly, therefore, [K+] did not correlate with Braak stage. The lack of change in [K+] is in good agreement with an earlier report [4] that [K+] is unchanged in the parietal cortex in AD although [K+] was elevated in the cerebellum [4]. Unexpectedly, however, we found that [K+] correlated positively with the Aβ plaque load, although it should be noted that plaque load data were available for only 32 brains (from only one of the three brain bank providers). Further work is needed to confirm this relationship and it would also be interesting to examine the relationship between [K+] and soluble forms of Aβ.

[Na+] and [K+] did not vary significantly with gender or age at death, with the single exception of a negative correlation between [K+] and age at death in DLB. As far as we are aware, this is the first time that either [K+] or [Na+] has been measured in postmortem tissue in DLB. We did not find overall [K+] or [Na+] to be significantly different in DLB from controls and the relationship with age may simply reflect a chance finding in a relatively small cohort but nonetheless merits further investigation.

A previous study found some correlation between brain [Na+] and [K+], with a tendency for control and AD samples to segregate in different areas of the graph when [K+] was plotted against [Na+] [4]. Our findings are partly supportive, in that severe AD samples tended to cluster toward the right hand side of the graph (see Fig. 1E). However, in our study the segregation was almost entirely due to differences in [Na+] and not [K+]. Only in the control group was there significant correlation between [Na+] and [K+], reflecting a balance between [Na+] and [K+] in the absence of any significant neurodegeneration.

[Na+] is influenced by synaptic activity and energy homeostasis in brain tissue. During synaptic transmission, glutamate receptor activation results in the influx of sodium ions into the cell. Sodium ion concentration is restored by the sodium pump, powered by ATP catabolism [14, 15]. Glucose is the predominant source of ATP in neurons. In AD impairment of glucose energy metabolism has been reported to be caused by a number of factors including mitochondrial dysfunction, alterations in mitochondrial mobility and reduced glucose uptake [16], all of which could potentially affect sodium ion homeostasis. Dysfunction of K+ channels was also reported in studies on fibroblasts from patients with AD [17] and mimicked in normal fibroblasts by treatment with a low concentration of $A\beta_{40}$ [18]. In human neuroblastoma cells, the overexpression of a deletion mutation (Δ9) in presenilin-1 led to a decreased K+ current [19]. Our data support a relationship between K+ channel dysfunction and Aβ accumulation, although the increase in [K+] in relation to plaque load may of course be indirect or a non-specific manifestation of other plaque-associated abnormalities [20].

In conclusion, we have found substantial elevation in [Na+] in Brodmann area 7 in severe AD and positive correlation of [Na+] with disease severity as measured by Braak tangle stage. In contrast, [K+] was lower in moderate but not severe AD, and correlated positively the Aβ plaque load.

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