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Alkali metals levels in the human brain tissue: Anatomical region differences and age-related changes

Patrícia Ramos[a], Agostinho Santos[b,c,d,e], Edgar Pinto[a,f], Nair Rosas Pinto[c], Ricardo Mendes[c], Teresa Magalhães[c,d,g], Agostinho Almeida[a,*]

[a] LAQV/REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, Porto University, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[b] National Institute of Legal Medicine and Forensic Sciences – North Branch, Jardim Carrilho Videira, 4050-167 Porto, Portugal
[c] CENCIFOR – Forensic Science Center, Largo da Sé Nova, s/n, 3000-213 Coimbra, Portugal
[d] Faculty of Medicine, Porto University, Al. Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
[e] School of Health Sciences, Minho University, Campus of Gualtar, 4710-057 Braga, Portugal
[f] CISA, Research Centre on Environment and Health, School of Allied Health Sciences, Polytechnic Institute of Porto, Rua Valente Perfeito 322, 4400-330 Vila Nova de Gaia, Portugal
[g] Institute of Research and Advanced Training in Health Sciences and Technologies (IINFACTS), Department of Sciences, University Institute of Health Sciences (IUCS), CESPU, R. Central da Gandra 1317, 4585-116 Gandra, Portugal

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ABSTRACT

The link between trace elements imbalances (both "toxic" and "essential") in the human brain and neurodegenerative disease has been subject of extensive research. More recently, some studies have highlighted the potential role of the homeostasis deregulation of alkali metals in specific brain regions as key factor in the pathogenesis of neurodegenerative diseases such as multiple sclerosis and Alzheimer's disease.

Using flame atomic emission spectrometry and inductively coupled plasma-mass spectrometry after microwave-assisted acid digestion of the samples, alkali metals (Na, K, Li, Rb and Cs) were determined in 14 different areas of the human brain (frontal cortex, superior and middle temporal gyri, caudate nucleus, putamen, globus pallidus, cingulated gyrus, hippocampus, inferior parietal lobule, visual cortex of the occipital lobe, midbrain, pons, medulla and cerebellum) of adult individuals (n = 42; 71 ± 12, range: 50–101 years old) with no known history and evidence of neurodegenerative, neurological or psychiatric disorder.

Potassium was found as the most abundant alkali metal, followed by Na, Rb, Cs and Li. Lithium, K and Cs distribution showed to be quite heterogeneous. On the contrary, Rb and Na appeared quite homogeneously distributed within the human brain tissue. The lowest levels of Na, K, Rb and Li were found in the brainstem (midbrain, medulla and pons) and cerebellum, while the lowest levels of Cs were found in the frontal cortex. The highest levels of K (mean ± sd; range 15.5 ± 2.5; 8.9–21.8 mg/g) Rb (17.2 ± 6.1; 3.9–32.4 μg/g and Cs (83.4 ± 48.6; 17.3–220.5 ng/g) were found in putamen. The highest levels of Na and Li were found in the frontal cortex (11.6 ± 2.4; 6.6–17.1 mg/g) and caudate nucleus (7.6 ± 4.6 2.2–21.3 ng/g), respectively.

Although K, Cs and Li levels appear to remain largely unchanged with age, some age-related changes were observed for Na and Rb levels in particular brain regions (namely in the hippocampus).

1. Introduction

Neurodegenerative diseases (ND), such as Alzheimer's and Parkinson's diseases, are chronic multifactorial diseases and it is assumed that they involve a complex interaction between (natural) ageing, and genetic and environmental factors [1–3]. Growing evidence indicates a role for oxidative stress [4–6] and some disturbances in transition metals levels (*e.g.*, Fe, Cu, Zn) [7,8]. More recently, imbalances in alkali metals levels (Na and K) have also been associated with some ND [9,10].

Potassium and Na are the most important cations of intra and extracellular fluids, respectively, playing a major role in many phys-

* Corresponding author.
E-mail address: aalmeida@ff.up.pt (A. Almeida).

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iological processes, such as electric impulse conduction in excitable cells (*e.g.*, neurons, cardiac cells) [11,12]. The concentration of these electrolytes is maintained by the Na^+/K^+-ATPase pump and imbalances of Na and K concentration in the extracellular fluid cause the movement of water into or out of the cells, altering the intracellular fluid osmotic pressure and causing cells swelling or shrinkage [13]. Osmotic shifts affect all cells and tissues but the brain is particularly susceptible to damage from changes in intracranial pressure [11].

Although Li does not have a known biological role and does not appear to be an essential element for humans, lithium salts (*e.g.*, carbonate, acetate) have been extensively used in the treatment of manic-depressive disorders for more than 50 years [14]. In the last two decades, several studies have further suggested that Li may also have neuroprotective effects against amyotrophic lateral sclerosis, and Alzheimer's and Parkinson's diseases [15].

Rubidium and Cs are also regarded as non-essential elements for humans and have no known biological role. In many animals, including humans, the distribution of both Rb and Cs resembles that of K and it has been shown that Rb and, to a lesser extent, Cs can replace K as an essential nutrient for the growth of bacteria, yeast and rats [16]. Although generally less readily transported, Cs has been shown to compete with K and Rb for both active and passive membrane transport due to their similar physicochemical properties [17].

It must be highlighted that the brain is a highly heterogeneous organ, with anatomically and physiologically very different areas, which may be affected in different manners by the ageing and neurodegenerative processes [18]. So, as a first step, a detailed study of the anatomical distribution of alkali metals in the "normal" brain is indispensable to clarify their role both in the normal human brain physiology and neurodegenerative diseases. To date, alkali metals (K, Na, Rb, Li and Cs) have been a poorly studied and data on their topographical distribution in the human brain are scarce, mostly limited to a few brain regions, to large or not specified brain regions, and/or involving a reduced number of subjects [19–22].

Based on this background, the main goal of the present study was to contribute to the establishment of reference levels for alkali metals in the different anatomical and functional regions of "normal" human brain. We directly determined Na, K, Rb, Cs and Li levels in postmortem human brain tissue in order to evaluate: (a) the regional anatomic differences across the brain and (b) their change in relation to age.

2. Materials and methods

2.1. Subjects

Brain samples were obtained from men (n = 27; 67 ± 11 years old) and women (n = 15; 77 ± 12 years old) not registered in the Portuguese *National Registry of Refusal to Organ Donation database* and complying with all the current regulations regarding human tissue collection for scientific research purposes.

Samples were collected from individuals submitted to forensic autopsy during the first semester of 2012 at the North Branch (Porto) of the Portuguese National Institute of Legal Medicine and Forensic Science (INMLCF). Individuals from each of the following age groups were studied: 50–59 ($n = 10$), 60–69 ($n = 10$), 70–79 ($n = 10$), 80–89 ($n = 9$) and ≥90 ($n = 3$) years old. Inclusion criteria were: (a) absence of known neurodegenerative, neurological or psychiatric disorder history; (b) absence of injuries involving CNS; (c) macroscopically normal brain tissue.

Samples from two individuals with documented Alzheimer's disease (women, 73 and 85 years old) and one individual with documented Parkinson's disease (woman, 91 years old) were also collected and results are also presented.

2.2. Sample collection

Samples were collected by INMLCF pathologists following a standard protocol. In order to prevent sample contamination, all materials contacting with the samples, including the stainless steel dissection tools, were previously decontaminated with 5% (v/v) nitric acid solution (prepared from concentrated HNO_3 ≥69%; Sigma-Aldrich, Germany) and thoroughly rinsed with ultrapure water (resistivity >18.2 MΩ cm at 25 °C), produced by an arium® pro (Sartorius, USA) water purification system.

After removing the brain from the cranium, the excess of blood was thoroughly washed with ultrapure water. Meninges were removed with plastic tweezers and the brain tissue was washed again with ultrapure water to minimize samples contamination with blood or cerebrospinal fluid.

In order to establish an accurate diagnosis and study the relationship between the disease process and either the clinical features seen in life or the cause of death, Paine and Lowe [18] have proposed a post-mortem approach where 14 key brain areas should be studied individually (Fig. 1). Using decontaminated plastic knives, tissue fragments (approximately 1 cm^3) were collected from the following brain areas: frontal cortex (1), superior (2A) and middle (2B) temporal gyri, caudate nucleus (3A), putamen (3B), globus pallidus (3C), cingulated gyrus (4), hippocampus (5), inferior parietal lobule (6), visual cortex of the occipital lobe (7), midbrain (including the *substantia nigra* at the level of the third nerve) (8), pons-locus coeruleus (9), medulla (10) and cerebellum-dentate nucleus (11). Samples were stored in decontaminated polypropylene tubes (Sarstedt, Germany) at −4 °C until analysis.

2.3. Sample pre-treatment

After thawing, brain samples were thoroughly washed with ultrapure water and placed in a dry oven (Raypa, Spain) at 110 °C until constant weight (ca. 24 h). Dried samples (ca. 100–500 mg) were weighed directly in the microwave digestion vessels previously washed with 10% (v/v) HNO_3 and rinsed with ultrapure water. Samples were digested with 2.5 mL of HNO_3 ≥65% and 1.0 mL of H_2O_2 ≥30% (both *Trace*SELECT® Ultra, Sigma-Aldrich, France). The digestion was performed in a MLS-1200 mega microwave oven (Milestone, Italy), equipped with a HPR 1000/10 rotor, using the following power (W)/time (min) program: 250/1, 0/2, 250/5, 400/5 and 600/5. After cooling, samples digests were made up to 50 mL with ultrapure water and stored in closed propylene tubes at 4 °C until analysis.

2.4. Potassium and sodium determination

Determination of K and Na was performed by flame atomic emission spectrometry (FAES), using a PerkinElmer (Germany) Model 3100 instrument according to the standard conditions recommended by the manufacturer (air/acetylene flame; K: 766.5 nm, slit 0.7 nm; Na: 589.0 nm, slit 0.2 nm).

Calibration curves were obtained with five standard solutions with concentrations ranging from 0.2 to 1.0 mg/L for Na and 0.4–2.0 mg/L for K, prepared by adequate dilution of 1000 mg/L single element commercial standard solutions (Sigma-Aldrich, Switzerland) with 0.2% v/v HNO_3. Sample solutions were also diluted (100-fold) with 0.2% v/v HNO_3. A 1% (w/v) Cs solution, prepared by dissolution of Cs_2CO_3 (Sigma-Aldrich, France) in ultrapure water, was added to all samples and calibration standards (final concentration: 0.1% (w/v)) in order to suppress analytes ionization.

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