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A post-mortem study of the anatomical region differences and age-related changes on Ca and Mg levels in the human brain

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ABSTRACT

Calcium and magnesium levels 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-locus coeruleus, medulla and cerebellum-dentate nucleus] of adult individuals ($n = 42$; 71 ± 12, range: 50–103 years old) without a known history of neurodegenerative, neurological or psychiatric disorder were studied.

Considering the mean values for the 14 regions, Mg was present at ca. 2.3-fold higher levels than Ca (mean ± sd: 527 ± 34 μg/g versus 226 ± 53 μg/g). Calcium distribution within the brain showed to be quite heterogeneous: highest levels were found in the occipital (306 ± 156 μg/g) and frontal cortex (287 ± 78 μg/g), while lowest levels were found in the medulla (186 ± 70 μg/g) and cerebellum (145 ± 42 μg/g). Higher Ca levels were found in women than in men (248 ± 59 μg/g versus 213 ± 46 μg/g; $p < 0.05$). A tendency for Ca levels to increase with age in all studied brain regions and in both genders was also observed. On the contrary, Mg presented a highly homogeneous distribution and seems to remain quite unchanged irrespective of aging.

1. Introduction

As a result of longer life expectancy, neurodegenerative diseases (ND) will become leading diseases worldwide during the next decades [1]. Besides "natural" aging, genetic predisposition and environmental factors [2], imbalances on major and trace element (both essential and toxic) homeostasis in particular areas of the brain have been identified as potentially responsible for the cognitive decline associated with normal aging and the development of some ND [3,4], but the evidence is still fragmentary and its definite role remains unclear.

Calcium (Ca) plays a central role in neuronal physiology. As a ubiquitous second messenger, Ca regulates energy production, gene expression, membrane excitability, dendrite development and synaptogenesis [5]. In particular, intracellular Ca ions play a major role in neurons as the trigger for neurotransmitter release [6], regulate neuronal plasticity underlying learning and memory and neuronal survival [7].

Besides being a critical cofactor in numerous enzymatic reactions, magnesium (Mg) is involved in several cellular processes, including ATP production, oxygen uptake, electrolyte balance, glucose metabolism, DNA transcription and protein synthesis [8]. Regarding the central nervous system, Mg has a neuroprotective role as a blocker of the N-methyl-D-aspartate (NMDA) receptor ion channel, which regulates Ca^{2+} transport through the plasma membrane and prevents ischemic damage to neurons. Since neural activity depends on the Mg concentration, when glutamate is released under anoxia conditions, it acts as a neuromodulator and protects cells from excitotoxicity [9].

Disturbances in the homeostasis of cytosolic Ca have been implicated in the pathogenesis of several acute and chronic ND and in brain aging [10–12]. Magnesium brain levels seem to be decreased in elderly people and some authors believe that such depletion, particularly in the hippocampus, may represent an important pathogenic factor of some ND [13,14].

Most of the current information about the relationship between metals and human brain functioning is based on animal studies [15,16] or relies on determinations in cerebrospinal fluid, whole blood or blood serum or plasma [15,17–21]. Studies on major metals (such as Ca and Mg) and trace element levels in normal and pathological human brains are scarce, limited to a few brain areas [22–24] and/or

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involve a small number of subjects [25,26]. Additionally, and regarding the specific topic of the distribution of metals in the brain, most of the published post-mortem [22,26,27] and in vivo [28,29] studies are focused solely on iron. Therefore, more extensive elemental mappings are needed in order to understand the specific roles of major biometals and trace elements in the human brain and to interpret the data obtained from ND patients.

Based on this background, the main goal of the present study was to directly quantify Ca and Mg levels in 14 different areas of the human brain in order to evaluate a) the regional anatomic differences and b) age-related changes in Ca and Mg levels. The results found in one Parkinson's disease and two Alzheimer's disease patients are also presented.

2. Materials and methods

2.1. Subjects

Brain samples were collected 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 obtained from individuals submitted to forensic autopsy exams during the first semester of 2012 at the North Branch (Porto) of the Portuguese National Institute of Legal Medicine (INML). 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) the absence of a history of known neurodegenerative, neurological or psychiatric disorders, b) the absence of injuries involving the central nervous system (CNS), and c) macroscopically normal tissues.

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

2.2. Sample collection

Samples were collected by the pathologists at the INML following a standard protocol.

To prevent sample contamination, all materials in contact with the samples, including the stainless steel tools used by the pathologists, were previously decontaminated with a 5% (v/v) nitric acid solution prepared from concentrated (≥69%) HNO_3 (Sigma-Aldrich, Germany) and thoroughly rinsed with ultrapure water (resistivity 18.2 MΩ·cm at 25 °C) produced by a Milli-Q water purification system (Millipore, USA).

After removing the brain from the cranium, the contaminating blood was washed away with ultrapure water. The meninges were removed with plastic tweezers, and the brain was washed again with ultrapure water to minimize sample contamination with blood or cerebrospinal fluid. Since each brain hemisphere is divided in four neuroanatomical and functional lobes, the function of the affected neurons determines the clinical features, which can be grouped into two main categories: cognitive impairment (e.g. Alzheimer's disease) and movement disorders (e.g. Parkinson's disease).

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 [30] have recently proposed a post-mortem approach where 14 key areas are suggested to be studied (Fig. 1). In this study, samples from those key areas were obtained.

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; basal ganglia, including the caudate nucleus (3A), putamen (3B) and 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 defrosting, the 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 decontaminated with 10% (v/v) HNO_3 and thoroughly rinsed with ultrapure water. Samples were digested with 2.5 mL of concentrated (≥65% m/m) HNO_3 (TraceSELECT®, Fluka, France) and 1.0 mL of ≥30% (v/v) H_2O_2 solution (TraceSELECT®, Fluka, Germany) in an MLS 1200 Mega microwave oven (Milestone, Italy), equipped with an HPR 1000/10 rotor, and using the following power (W)/time (min) program: 250/1, 0/2, 250/5, 400/5 and 600/5. After cooling, sample solutions were made up to 50 mL with ultrapure water and stored in closed propylene tubes at 4 °C until analysis.

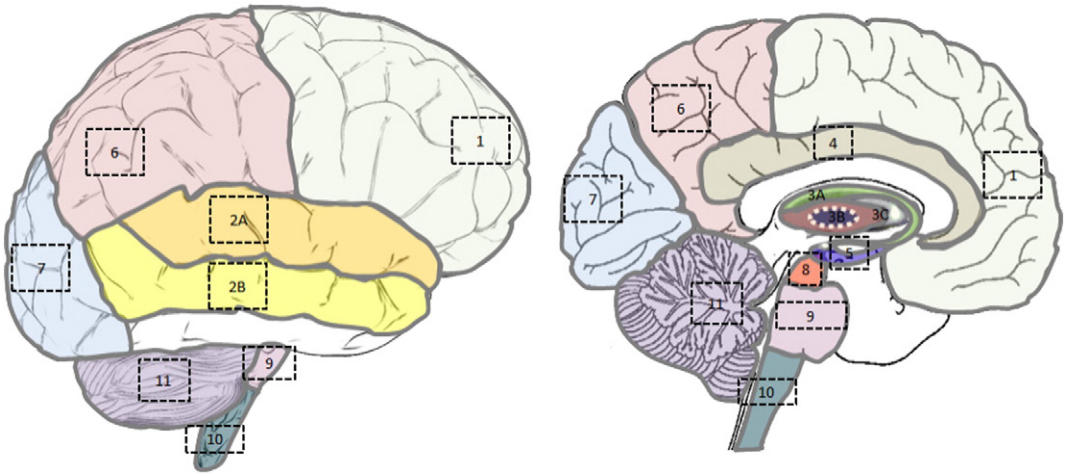

Fig. 1. Sampled brain regions, as suggested by Paine and Lowe [25]: frontal cortex (1), superior (2A) and middle (2B) temporal gyri, basal ganglia including the caudate nucleus (3A), putamen (3B) and globus pallidus (3C), cingulated gyrus (4), hippocampus (5), inferior parietal lobule (6), visual cortex 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).