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Deregulation of purine metabolism in Alzheimer's disease

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ABSTRACT

The neuroprotective role of adenosine and the deregulation of adenosine receptors in Alzheimer's disease (AD) have been extensively studied in recent years. However, little is known about the involvement of purine metabolism in AD. We started by analyzing gene expression in the entorhinal cortex of human controls and AD cases with whole-transcript expression arrays. Once we identified deregulation of the cluster purine metabolism, messenger RNA expression levels of 23 purine metabolism genes were analyzed with qRT-PCR in the entorhinal cortex, frontal cortex area 8, and precuneus at stages I-II, III-IV, and V-VI of Braak and Braak and controls. APRT, DGUOK, POLR3B, ENTPD3, AK5, NME1, NME3, NME5, NME7, and ENTPD2 messenger RNAs were deregulated, with regional variations, in AD cases when compared with controls. In addition, liquid chromatography mass spectrometry based metabolomics in the entorhinal cortex identified altered levels of dGMP, glycine, xanthosine, inosine diphosphate, guanine, and deoxyguanosine, all implicated in this pathway. Our results indicate stage- and region-dependent deregulation of purine metabolism in AD.

1. Introduction

Alzheimer's disease (AD) is a human age-related biological process which causes progressive degeneration of the brain and which is characterized clinically by cognitive impairment and dementia, and neuropathologically by the variable combination of 2 hallmarks, neurofibrillary tangles composed of abnormally phosphorylated, and conformed and truncated tau, and senile plaques with a core of the altered cleavage of amyloid precursor protein leading to the deposition of β-amyloid (Duyckaerts and Dickson, 2011; Haass and Selkoe, 2007; Lowe et al., 2008). In addition to the pathologic hallmarks of AD, several other abnormalities have been reported in AD, including mitochondrial malfunction; increased oxidative stress and oxidative/nitrosative damage to nucleic acids, proteins, and lipids; energy metabolism failure; altered composition of lipids in lipid rafts; neuroinflammation; and endoplasmic reticulum stress, impaired ubiquitin-proteasome system, and autophagy (Ferrer, 2012). Multiple signaling and neurotransmitter pathways are altered compromising neuron-neuron interactions and glial-neurons interactions.

Purines are heterocyclic double-ring aromatic organic molecules; primary purine adenine and guanosine nucleobases, together with one-ring primary pyrimidine nucleobases cytosine, thymidine, and uracil, are the core of DNA, RNA, nucleosides, and nucleotides. Adenosine and guanosine are purine ribonucleosides resulting from the β-N9-glycosidic bound to adenine or guanine and ribose, respectively. When adenine and guanine are attached to the C1 carbon of a deoxyribose ring, the resulting compounds are deoxyadenosine and deoxyguanosine, respectively. Nucleotides result from the incorporation of phosphate groups in nucleosides: Adenosine monophosphate (AMP), adenosine diphosphate, Adenosine triphosphate (ATP), guanosine monophosphate, guanosine diphosphate, guanosine triphosphate, and cyclic forms cAMP and cGMP are primary purine-derived nucleotides. Modified purine nucleobases hypoxantine and xanthine result from the replacement of the amino-group by a carbonyl-group from adenine and guanine, respectively, whereas methyl-guanine results from the incorporation of a methyl

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group to guanine. Corresponding modified purine nucleosides are inosine, xanthosine, and methyl-guanosine, respectively. Nucleotides participate in a wide variety of crucial metabolic pathways including energy metabolism and cell signaling. In addition, purine bases are incorporated to other molecules to form cofactors of several enzymatic reactions such as coenzyme A, flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD+), nicotinamide adenine dinucleotide phosphate (NADP+), and the corresponding reduced forms FADH2, NADH, and NADPH. S-Adenosyl methionine is made from ATP and methionine by methionine adenosyltransferase and is involved in the transfer of methyl groups to distinct substrates, including nucleic acids, proteins, lipids, and metabolites. In addition to intracellular signaling, purines and their products may function as extracellular signals acting on other cells, either between neurons or neurons and glial cells equipped with appropriate receptors (Ipata et al., 2011). Adenosine is responsible for regulating, integrating and fine-tuning neuronal activity, and influencing relevant brain functions, including sleep and arousal, cognition and memory, and neuronal damage and degeneration, by acting as an extracellular molecular via specific adenosine receptors (Rahman, 2009). Adenosine receptor signaling also modulates permeability of the blood-brain barrier (Carman et al., 2011). Adenosine exerts its function by binding adenosine receptors which are G-protein-coupled receptors (A1, A2A, A2B, and A3) that can inhibit (A1 and A3) or enhance (A2) neuronal communication through neurotransmitter release (Ribeiro et al., 2003). Several indirect data suggest altered purine metabolism in AD. Energy metabolism dependent on mitochondrial function and ATP production is markedly altered in AD (Ferreira et al., 2010; Ferrer, 2009). In addition, oxidative damage to DNA and RNA, as revealed by the increase in 8-hydroxyguanosine, is found in the brain in AD (Lovell and Markesbery, 2008; Lovell at al., 2011; Markesbery and Lovell, 2006; Nonumura et al., 2012; Weidner et al., 2011). Regarding purine neurocrine factors, adenosine receptors have attracted interest because of the neuroprotective effects of adenosine in several neurologic disorders (Boisson, 2008; Ribeiro et al., 2003). Experiments carried out with cell lines have demonstrated that A1 activation mediates the production of soluble forms of β-amyloid and tau phosphorylation (Angulo et al., 2003). Furthermore, blockade of A2A receptors in rat cultured cerebellar granule neurons with Aβ25-35 prevents β-amyloid-induced neurotoxicity (Dall'Igna et al., 2003), suggesting that the presence of A2A is critical for Aβ toxicity (Rahman, 2009). cAMP is an endogenous modulator of the β-amyloid precursor protein metabolism (Canepa et al., 2013). Decreased levels of A1 receptors and altered binding of adenosine agonists and antagonists to A1 receptors have been observed in the molecular layer of the CA1 region of the hippocampus in AD (Ulas et al., 1993). In contrast, upregulation of A1 and A2A receptors has been reported in the frontal cortex in AD (Albasanz et al., 2008).

Direct alterations of purine metabolism in AD have also been detected by metabolomics in the ventricular cerebrospinal fluid at postmortem (Kaddurah-Daouk et al., 2011) and in the cerebrospinal fluid in living individuals (Isobe et al., 2010; Kaddurah-Daouk et al., 2013; Jové et al., 2014). Only a limited number of metabolomic studies have been carried out in AD brains (Jové et al., 2014).

However, little is known about possible alterations in the expression of genes encoding enzymes involved in purine metabolism in AD brains. This information would permit a better understanding of the primary regulation of purine-related genes and their possible implications in the pathogenesis of the disease. First, functional genomics in a short series of AD cases revealed a deregulated cluster of genes involved in purine metabolism in the entorhinal cortex in AD brains. Second, the expression of selected genes was analyzed in the enthorinal cortex, frontal cortex area 8, and precunneus at stages I-II, III-IV, and V-VI of Braak and Braak, compared with controls. Finally, metabolomics was applied to the study of the entorhinal cortex to increase the understanding of functional implications of deregulated purine metabolism. We fully understand that the postmortem study of the human brain has certain limitations because it cannot be manipulated premortem, and because it is subject to degradation processes. Nevertheless, the study of the human postmortem brain cannot be replaced by any model, and it represents an invaluable source of knowledge.

2. Methods

2.1. Human cases

Brain tissue was obtained from the Institute of Neuropathology HUB-ICO-IDIBELL Biobank following the guidelines of Spanish legislation on this matter and the approval of the local ethics committee. The postmortem interval between death and tissue processing was between 3 and 20 hours. One hemisphere was immediately cut in coronal sections, 1-cm thick, and selected areas of the encephalon were rapidly dissected, frozen on metal plates over dry ice, placed in individual air-tight plastic bags, numbered with water-resistant ink, and stored at −80 °C until use for biochemical studies. The other hemisphere was fixed by immersion in 4% buffered formalin for 3 weeks for morphologic studies. Neuropathologic study in all cases was routinely performed on 20 dewaxed paraffin sections comprising different regions of the cerebral cortex, diencephalon, thalamus, brain stem, and cerebellum, which were stained with hematoxylin and eosin, Klüver-Barrera, and for immunohistochemistry to microglia, glial fibrillary acidic protein, β-amyloid, phosphorylated tau (clone AT8), α-synuclein, TDP-43, ubiquitin, and p62.

Neuropathologic diagnosis of AD was based on the classification of Braak and Braak (Braak and Braak, 1991, 1999) adapted to paraffin sections (Braak et al., 2006).

Cases with combined pathologies (i.e., Parkinson's disease, tauopathy, vascular diseases, and metabolic syndrome) were excluded from the present study. Age-matched control cases had not suffered from neurologic or psychiatric diseases, or metabolic diseases (including metabolic syndrome), and did not have abnormalities in the neuropathologic examination (excepting Braak and Braak stages I-II).

Cases used in the present study for messenger RNA (mRNA) expression corresponded to 23 AD stages III-IV (12 men, 11 women), 35 AD stages V-VI (20 men, 15 women), and 34 controls (21 men, 13 women). Three regions were examined: entorhinal cortex, frontal cortex, and precuneus. However, whole-transcript expression arrays were carried out in the entorhinal cortex in 5 controls and 5 AD cases stage V-VI. These cases were accepted once the RNA integrity number (RIN) values were considered sufficient to perform RNA studies. A summary of the cases used for mRNA studies are shown in Table 1.

In addition to these cases, AD samples (n = 16) of the entorhinal cortex stages from stages ranging AD I-II (3 women and 4 men, aged between 55 and 79 years), AD III-IV (2 men and 2 women, aged between 68 and 80 years), and V-VI (1 women and 4 men, aged between 75 and 87 years) in comparison with control cases (n = 4) were added for metabolomics studies. The postmortem delay in all these cases was between 3 hours 45 minutes and 9 hours 30 minutes, and particular attention was paid to include cases with short or sudden agonic state, renal, hepatic, and respiratory insufficiency, infections and neoplasia, in addition to those criteria referred previously.

2.2. Whole-transcript expression arrays

RNA samples from entorhinal cortex of control and AD cases were analyzed using the Affymetrix microarray platform and the Genechip Affymetrix Human Exon 1.0 ST Array. This array analyzes