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The Protective Effects of IGF-I against β-Amyloid-related Downregulation of Hippocampal Somatostatinergic System Involve Activation of Akt and Protein Kinase A

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Abstract—Somatostatin (SRIF), a neuropeptide highly distributed in the hippocampus and involved in learning and memory, is markedly reduced in the brain of Alzheimer's disease patients. The effects of insulin-like growth factor-I (IGF-I) against β amyloid (Aβ)-induced neuronal death and associated cognitive disorders have been extensively reported in experimental models of this disease. Here, we examined the effect of IGF-I on the hippocampal somatostatinergic system in Aβ-treated rats and the molecular mechanisms associated with changes in this peptidergic system. Intracerebroventricular Aβ25-35 administration during 14 days (300 pmol/day) to male rats increased Aβ25-35 levels and cell death and markedly reduced SRIF and SRIF receptor 2 levels in the hippocampus. These deleterious effects were associated with reduced Akt and cAMP response element-binding protein (CREB) phosphorylation and activation of c-Jun N-terminal kinase (JNK). Subcutaneous IGF-I co-administration (50 μg/kg/day) reduced hippocampal Aβ25-35 levels, cell death and JNK activation. In addition, IGF-I prevented the reduction in the components of the somatostatinergic system affected by Aβ infusion. Its co-administration also augmented protein kinase A (PKA) activity, as well as Akt and CREB phosphorylation. These results suggest that IGF-I co-administration may have protective effects on the hippocampal somatostatinergic system against Aβ insult through up-regulation of PKA activity and Akt and CREB phosphorylation.

Key words: Alzheimer's disease, β-amyloid, hippocampus, IGF-I, protein kinase A, somatostatin.

INTRODUCTION

One of the histological characteristics of Alzheimer's disease (AD) is the accumulation of senile plaques in brain tissue, with the principal component of these plaques being β-amyloid (Aβ), a protein involved in the pathogenesis of AD (Tomita, 2017). This disease, the most common cause of cognitive function decline, is also characterized by increased neuronal death (Shi et al., 2016). One of the most common alterations of a neurotransmitter in the brain and cerebrospinal fluid of AD patients is a decline in somatostatin (SRIF) (Molchan et al., 1993; Nilsson et al., 2001), which is widely distributed in the hippocampus, an area involved in cognitive function control and where SRIF-containing neurons are implicated in physiological functions (Martel et al., 2012). SRIF neurons comprise a subpopulation of GABAergic interneurons, involved in the innervation of

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Abbreviations: AC, adenylyl cyclase; AD, Alzheimer's disease; Akt, protein kinase B; Aβ, beta-amyloid; BDNF, brain-derived neurotrophic factor; CCK, cholecystokinin, CRE, cyclic AMP response element; CREB, CRE-binding protein; GABA, γ-aminobutyric acid; GFAF, glial fibrillary acidic protein; GSK3β, glycogen synthase kinase 3β; HRP, horseradish peroxidase; Iba1, ionized calcium-binding adaptor molecule 1; ICV, intracerebroventricular; IDE, insulin-degrading enzyme; IGF-I, insulin-like growth factor I; IL, interleukin; IRS1, insulin receptor substrate 1; JNK, c-Jun N-terminal kinase; MBIA, multiplexed bead immunoassay; NeuN, neuronal nuclei; NPY, neuropeptide Y; NT-3, neurotrophin-3; p, phosphorylated; PI3K, phosphatidylinositol-3 kinase; PKA, protein kinase A; RIA, radioimmunoassay; SRIF, somatostatin; SRIF-LI, SRIF-like immunoreactivity; SST, SRIF receptor subtype; TTBS, Tris–Tween-buffered saline; VIP, vasoactive intestinal peptide.

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other neurons (Katona et al., 2014). Other neurotransmitter systems, such as the cholinergic and mainly the noradrenergic systems are severely disturbed in the brain of AD patients, and interestingly; degeneration of the noradrenergic system could be associated with alterations in somatostatin receptor 2 expression (Ádori et al., 2015).

Reduced SRIF levels are associated to memory deficit (Gahete et al., 2010), with a close correlation between Aβ levels and brain SRIF deficiency in an experimental model of AD disease (Ramos et al., 2006). Moreover, the decrease in the cerebrospinal fluid SRIF levels of patients with AD correlates with the severity of dementia (Minthon et al., 1997). The actions of SRIF in the hippocampus are mediated via specific transmembrane receptors coupled to adenylyl cyclase (AC) (Lahlou et al., 2004). Receptors for SRIF (sst) are widely distributed in the hippocampus and sst-expressing cells are interconnected with neurons that produce key neurotransmitters, reinforcing the idea that SRIF plays a role in hippocampal functions, such as motor activity and cognition (Viollet et al., 2008).

Infusion of Aβ is accepted as an experimental approach to AD as it induces deposits of Aβ in the hippocampus associated with neuronal loss, deficits in synaptic plasticity, a decline in several neurotransmitters and learning impairment (Nguyen et al., 2007; Ludka et al., 2016). Intracerebroventricular (ICV) infusion of Aβ reduces brain SRIF content (Nag and Tang, 2001; Aguado-Llera et al., 2007), with the Aβ25-35 fragment exerting a more pronounced deleterious effect than Aβ1-42 on SRIF levels (Aguado-Llera et al., 2005). Indeed, the toxic effect of Aβ has been assigned to this fragment that has a domain for aggregation (Kubo et al., 2002).

Different approaches have been investigated in an attempt to block the deleterious effects of Aβ and to lessen the progression of this disease. Insulin-like growth factor-I (IGF-I) acts as a survival factor in the brain through activation of the Akt pathway (Wang et al., 2015). Some neurodegenerative conditions have reduced levels of IGF-I in plasma and brain, both in humans (Torres-Alemán et al., 1998) and in AD experimental models (Busiguina et al., 2000), suggesting that this growth factor may be implicated in the etiopathogenesis of AD. Furthermore, IGF-I reduces brain Aβ content (Carro et al., 2002) and increases hippocampal neurogenesis, improving memory performance (Pardo et al., 2016).

Cyclic AMP (cAMP) could mediate some protective effects as local increases in the hippocampus correlate with the activation of protein kinase B (Akt) and cAMP response element-binding protein (CREB), which is associated with maintenance of neuronal structural integrity and cognitive function (Sun et al., 2005). In this regard, when low levels of cAMP in patients with learning difficulties and memory loss are increased, these cognitive alterations are significantly alleviated (Balakrishnan et al., 2016). In fact, the protein kinase A (PKA)/CREB pathway plays a key role in learning and pharmacological activation of PKA improves memory loss in an experimental model of AD (Kumar and Singh, 2017).

These previous findings indicate that IGF-I could have beneficial effects in AD. However, there is little information available regarding the effectiveness of IGF-I in protecting against Aβ-induced damage of specific neurotransmitter systems involved in the regulation of learning and memory. Therefore, this study investigated whether continuous IGF-I administration could prevent the deleterious changes in the somatostatinergic system provoked by Aβ25-35 infusion in the rat hippocampus (Burgos-Ramos et al., 2008). Thus, we analyzed SRIF-like immunoreactivity (SRIF-LI), the levels of the SRIF receptor subtypes (sst) 1-4 and the main adenylyl cyclase (AC) subtypes in the rat hippocampus after chronic Aβ administration in the absence or presence of continuous IGF-I infusion. In addition, we examined the effect of IGF-I on hippocampal Aβ25-35 content, cell death and the relationship with possible changes in activation of IGF-I-related pathways with those parameters affected by Aβ infusion. We studied different neuropeptides expressed in hippocampal interneurons as controls for the specificity of Aβ and/or IGF-I effects on SRIF neurons. We also analyzed the effect of these treatments on the number of hippocampal somatostatinergic neurons and the content of various anti-inflammatory interleukins (IL) in the hippocampus. Finally, since several Aβ proteases are modulated by SRIF levels (Burgos-Ramos et al., 2009; Tundo et al., 2012), the concentrations of neprilysin and insulin-degrading enzyme (IDE) in the hippocampus were also determined.

EXPERIMENTAL PROCEDURES

Experimental animals

Twenty-five male Wistar rats weighing 240 ± 10 g were divided into five groups of five animals each. Rats were anesthetized (0.08 ml of ketamine/100 g wt and 0.04 ml/100 g wt of xylazine) and a cannula fixed to an osmotic minipump (Alzet 2002, Alza, Palo Alto, CA, USA) implanted into the right cerebral ventricle (−0.3 mm anteroposterior, 1.1 mm lateral). Aβ25-35 was infused for 14 days (300 pmol/day, infusion rate 0.5 μL/h), as described (Nag and Tang, 2001). A second group received Aβ25-35, simultaneously with IGF-I via a subcutaneous osmotic minipump (Alzet 2002; 50 μg/kg/day, rate 0.5 μL/h). Another group of rats received a continuous i.c.v. infusion of vehicle and IGF-I at the same dose as above via the implanted subcutaneous minipump. Control rats received vehicle and a saline infusion by the same administration routes. Lastly, to compare the toxicity of Aβ25-35 and Aβ1-42, the last group of rats received Aβ1-42 at the same dose, route and time-period as the Aβ25-35 group. After treatment, the rats were sacrificed and the hippocampus was dissected (Glowinski and Iversen, 1966). The rats were treated according to the European Community laws for animal care and the experiment was approved by the Animal Care Committee of Alcalá University.

Tissue homogenization and protein quantification

For immunodetection of AC I, AC V/VI, AC VIII, Akt, CREB, glial fibrillary acidic protein (GFAP), glycogen synthase kinase (GSK)3β, ionized calcium-binding