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Deregulation of hypothalamic-pituitary-adrenal axis functions in an Alzheimer's disease rat model

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ABSTRACT

Elevated cortisol evidence in Alzheimer's disease (AD) patients prompted the hypothesis that stress and glucocorticoids are involved in the development and/or maintenance of AD. We investigated the hypothalamic-pituitary-adrenal (HPA) axis activity, functionality, and reactivity for up to 6 weeks after an intracerebroventricular injection of amyloid-β_{25-35} peptide ($A\beta_{25-35}$) in rat, a validated acute model of AD. $A\beta_{25-35}$ induces memory impairment, alteration of anxiety responses, HPA axis hyperactivity, and glucocorticoid (GR) and mineralocorticoid (MR) receptor increases in brain regions related to HPA axis functions. GR are progressively translocated in neurons nucleus, while membrane version of MR is evidenced in all structures considered. The MR/GR ratio was modified in all structures considered. $A\beta_{25-35}$ induces a subtle disturbance in the feedback of the HPA axis, without modifying its functionality. The reactivity alteration is long-lasting, suggesting that amyloid toxicity affects the HPA axis adaptive response to stress. These findings are evidence of progressive HPA axis deregulation after $A\beta_{25-35}$, which is associated with an imbalance of MR/GR ratio and a disruption of the glucocorticoid receptors nucleocytoplasmic shuttling, and suggest that elevated glucocorticoids observed in AD could be first a consequence of amyloid toxicity.

1. Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative pathology characterized by the presence in the brain of senile plaques and neurofibrillary tangles accompanied by synaptic and neuronal loss (Selkoe, 1999). The major component of senile plaques is an amyloid-β protein (Aβ). In AD patients, cognitive deficits (such as memory) and psychological symptoms (such as anxiety) are associated with an early deregulation of the hypothalamo-pituitary-adrenal (HPA) axis (Swanwick et al., 1998). Deregulation is also the most prevalent and well documented neuroendocrine abnormality in stress-related disorders and particularly in depression (Holsboer and Barden, 1996).

The HPA axis, which is highly involved in the stress response, triggers the adrenal cortex to release glucocorticoids. These steroid hormones readily cross the blood–brain barrier and bind to low affinity glucocorticoid (GR) and high affinity mineralocorticoid (MR) receptors (Reul and de Kloet, 1985). These nuclear receptors are necessary for normal cellular activity and crucial for many central nervous system functions, including learning and memory (Roozendaal, 2000). Because glucocorticoids act synergistically with excitatory amino acids, and particularly glutamate, disturbances of the HPA axis could be extremely toxic, especially in the hippocampus (McEwen, 2008). They could contribute to the cognitive decline and psychological symptoms that occur in AD and thus participate in its etiology.

The hippocampus and amygdala are relevant structures of the limbic system that are highly involved in cognitive and psychological functions. They are also important components of neural circuitry mediating HPA axis activity (Jankord and Herman, 2008). Though structural plasticity in the hippocampus might mediate cognitive impairment caused by severe stress, changes in amygdala are more likely to contribute to the affective aspect of stress disorders (Vyas et al., 2004). Recently, we obtained evidence that these 2 structures were rapidly and extremely affected by a single intracerebroventricular (icv) injection of an Aβ fragment ($A\beta_{25-35}$) in rats (Zussy et al., 2011).

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In rats, $A\beta_{25-35}$ induced a wide pattern of central modifications reminiscent of the human physiopathology, particularly short- and long-term memory deficits, oxidative stress, apoptosis, neuroinflammation, acetylcholine impairment, hippocampus alteration, tau hyperphosphorylation, and amyloid burden (Klementiev et al., 2007; Zussy et al., 2011). No data are available on the effects of Aβ icv injection on HPA axis activity, functionality, and reactivity. By contrast, several studies have demonstrated that glucocorticoids modulate amyloid processing (Catania et al., 2009; Green et al., 2006), increase Aβ-induced neurodegeneration of cholinergic neurons (Abraham et al., 2000), and $A\beta_{25-35}$ toxicity in hippocampus neurons (Goodman et al., 1996), thus reinforcing the hypothesis that stress and glucocorticoids might contribute to the development and/or maintenance of AD. Moreover, in transgenic mouse lines, stress and glucocorticoid administration affect the course of the pathology. Chronic behavioral stress enhanced plaque pathology and accelerated the onset of cognitive deficits in Tg2576 and APP-CT100 mice (Dong et al., 2004; Jeong et al., 2006). In 3xTg AD mice, chronic stress exacerbated Aβ accumulation and impaired neurotrophic signaling (Rothman et al., 2012). In APP/PS1 mouse, social isolation exacerbated the impairment of spatial working memory associated with an increase of $A\beta_{1-42}$ in the hippocampus (Huang et al., 2011). In AD patients, stress-related psychiatric disorders (e.g., anxiety and depression) have been identified as a risk for developing AD (Caraci et al., 2010; Ownby et al., 2000, 2006).

This study aims to determine the time-course effect of amyloid toxicity on HPA axis activity, functionality, and reactivity. In addition, in relation with the HPA axis regulation and to complete previous studies on the behavior effects of amyloid toxicity in rats (Zussy et al., 2011), we therefore characterized the cognitive and anxious states of control, scrambled $A\beta_{25-35}$ peptide-, and $A\beta_{25-35}$ peptide-injected rats after 1, 2, 3, and 6 weeks using novel object recognition and elevated plus-maze procedures. To characterize the activity of the HPA axis, we determined temporal variations in the hypothalamic corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) contents, plasma adrenocorticotropin hormone (ACTH), and corticosterone concentrations. The level, ratio, and cellular localization of MR and GR variations were determined in the amygdala, hippocampus, and hypothalamus. To evaluate the functionality of the HPA axis, the sensitivity to glucocorticoid-mediated negative feedback was tested using the synthetic glucocorticoid dexamethasone (DEX). Finally, to characterize HPA axis reactivity, the animals were subjected to forced swimming stress.

2. Methods

2.1. Animals

Adult male Sprague-Dawley rats (Depré, France) weighing 260–280 g (6–7 weeks) at the beginning of the experiments were individually housed in a standard animal facility (12-hour light/dark cycle, 21 ± 1 °C, food and water ad libitum). The animals were treated in accordance with the European Community Council Directive 86/609, modified by the decrees 87/848 and 2001/464. The Animal Welfare Committee at the University of Montpellier 2 approved all protocols and all efforts were made to minimize the number of animals used and potential pain and distress. All surgery was performed under ketamine/xylazine mixture, and all efforts were made to minimize suffering. All experiments were performed between 9 AM and 2 PM (i.e., during the diurnal part of the HPA axis rhythm).

2.2. Aβ peptide

$A\beta_{25-35}$ and scrambled $A\beta_{25-35}$ peptides (NeoMPS) were solubilized in sterile water at 1 μg/μL concentration and stored at −20 °C. The $A\beta_{25-35}$ and scrambled peptides were aggregated by in vitro incubation at 37 °C for 4 days (Maurice et al., 1996).

2.3. Experimental procedures

This study was divided into 4 sections (behavioral, HPA axis activity, functionality, and reactivity). The animals were divided into 3 groups. One group was left undisturbed (control group), the second received an icv injection of incubated scrambled peptide (10 μg per rat; scrambled group) and the third received an icv injection of aggregated $A\beta_{25-35}$ (10 μg per rat; $A\beta_{25-35}$ group). Control animals were housed in the same conditions and time as treated animals. The icv injection into the lateral ventricles was performed in anesthetized animals with an intramuscular injection of a ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture, and using a stereotaxic apparatus (coordinates: anterior-posterior, −1 mm; Lateral, ± 1.5 mm; dorsal-ventral, −3.5 mm) (Paxinos and Watson, 1997). Animals used for behavioral tests were submitted to only 1 test and were not used for any other analyses.

2.4. Behavioral procedures

The novel object recognition test assesses recognition memory and takes advantage of rodents' innate behavior to spend more time investigating a novel object than one previously encountered (Eichenbaum et al., 2007). The apparatus consists of a squared open field (1 m^2 × 0.6 m high) made of white polyvinyl-chloride (PVC) with an infrared light emitting floor. An infrared sensitive charge-coupled device (CCD) camera was placed above the field and connected to a videotracking system (ViewPoint). The test consists of 3 sessions separated by 24 hours. In session 1 (habituation), animals were allowed to freely explore the open field during 10 minutes. In session 2 (familiarization), rats were allowed to interact with 2 identical objects placed in the center of the open field during two 5-minute periods. During interperiod time (1 hour), rats were placed in their home cage. In session 3 (test), rats were presented 1 familiar object and a novel object that differed in shape, color, and texture, during a 10-minute session (Meunier et al., 2006). The starting position (facing objects) was unchanged over sessions and animals were tested in a semirandomized order. Contacts with objects were defined as when the animal's nose was less than 1 cm from the object and contacts were recorded using the Nosetrack software (Viewpoint). The results were calculated as duration of contact with the novel object and expressed as a percentage of the total contact time with the 2 objects (TCT). The apparatus was cleaned with diluted ethanol (50%) between animals and only 1 rat was tested by session.

Anxious state of rats was measured using their ability to explore open and enclosed arms of an elevated plus-maze (Espallergues et al., 2009). The clear plexiglass apparatus consisted of 2 open arms (50 × 10 cm) and 2 enclosed arms (50 × 10 × 45 cm high), extending from a central platform and placed 50 cm above the floor. Each rat was placed at the center of the plus-maze face closed arm and its exploration behavior was recorded for 10 minutes. The results were expressed as total time spent in the open arms and the total number of entries was counted to verify general motor activity. An entry into an arm was recorded if the animal crossed the line that connected that arm with the central platform with all 4 legs. The apparatus was cleaned between animals with diluted ethanol (50%).

2.5. AVP and CRH contents

After decapitation, the hypothalamus was immediately frozen on liquid nitrogen and stored at −20 °C until assay. CRH and AVP