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Allopregnanolone levels are reduced in temporal cortex in patients with Alzheimer's disease compared to cognitively intact control subjects

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ABSTRACT

The neurosteroid allopregnanolone has pronounced neuroprotective actions, increases myelination, and enhances neurogenesis. Evidence suggests that allopregnanolone dysregulation may play a role in the pathophysiology of Alzheimer's disease (AD) and other neurodegenerative disorders. Our prior data demonstrate that allopregnanolone is reduced in prefrontal cortex in male patients with AD compared to male cognitively intact control subjects, and inversely correlated with neuropathological disease stage (Braak and Braak). We therefore determined if allopregnanolone levels are also reduced in AD patients compared to control subjects in temporal cortex, utilizing a larger set of samples from both male and female patients. In addition, we investigated if neurosteroids are altered in subjects who are APOE4 allele carriers. Allopregnanolone, dehydroepiandrosterone (DHEA), and pregnenolone levels were determined in temporal cortex postmortem samples by gas chromatography/mass spectrometry, preceded by high performance liquid chromatography (40 subjects with AD/41 cognitively intact control subjects). Allopregnanolone levels are reduced in temporal cortex in patients with AD (median 2.68 ng/g, $n=40$) compared to control subjects (median 5.64 ng/g, $n=41$), Mann–Whitney $p=0.0002$, and inversely correlated with Braak and Braak neuropathological disease stage (Spearman $r=-0.38$, $p=0.0004$). DHEA and pregnenolone are increased in patients with AD compared to control subjects. Patients carrying an APOE4 allele demonstrate reduced allopregnanolone levels in temporal cortex (Mann–Whitney $p=0.04$). In summary, our findings indicate that neurosteroids are altered in temporal cortex in patients with AD and related to neuropathological disease stage. In addition, the APOE4 allele is associated with reduced allopregnanolone levels. Neurosteroids may be relevant to the neurobiology and therapeutics of AD.

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1. Introduction

Allopregnanolone is a neurosteroid with a number of properties that may be relevant to the pathophysiology and treatment of Alzheimer's disease (AD) and other neurodegenerative disorders, demonstrating pronounced neuroprotective actions in the setting of excitotoxicity [1,2], traumatic brain injury (TBI) [3–5], and neurodegeneration [6–8]. It also increases myelination [9–11], enhances neurogenesis [12], decreases inflammation [11,13,14], and reduces apoptosis [15–17]. Since excitotoxicity [18–21], neurodegeneration [22,23], and traumatic brain injury [24,25], as well as dysregulation in myelination [26,27], neurogenesis [28], apoptosis [29,30], and inflammation [31] have been implicated in the pathogenesis and clinical course of AD, deficits in allopregnanolone and/or alterations in its regulation could represent critical components of AD pathophysiology.

Emerging evidence demonstrating allopregnanolone deficits in neurodegenerative disorders is consistent with this hypothesis. For example, allopregnanolone levels are decreased in Niemann–Pick type C mice [7], a neurodegenerative disorder that shares a number of properties with AD. These include cholesterol dysregulation, neurofibrillary tangle formation, β-cleaved amyloid precursor protein accumulation, and myelin breakdown [28,32–37]. Further, allopregnanolone administration delays neurological symptom onset and doubles lifespan in Niemann–Pick type C mice [6–8]. Also consistent with a role for allopregnanolone in disorders in which neurodegeneration is a salient characteristic, allopregnanolone and other neurosteroids are altered in AD. Our laboratory determined previously that allopregnanolone

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levels in prefrontal cortex are significantly decreased in male AD patients compared to male cognitively intact control subjects, and that allopregnanolone levels are inversely correlated with neuropathological disease stage (Braak and Braak) [38]. Additional data also support the hypothesis that there may be allopregnanolone deficits in AD; for example, allopregnanolone is reduced in the periphery in serum [39] and plasma [40] in patients with AD compared to control subjects. Importantly, these earlier serum and plasma investigations also raise the possibility that other GABAergic neurosteroids (i.e. 3α-hydroxy-4-pregnen-20-one, [41,42]) with considerable cross-reactivity with the antibody used in radioimmunoassay procedures [40,43] may also be altered in AD.

It is possible that the determination of peripheral neurosteroid levels in blood may have proxy or surrogate biomarker potential for central neurosteroid levels in brain. Our prior efforts demonstrating that serum pregnenolone levels are closely correlated with hippocampal pregnenolone levels in rodents support this possibility [44]. Further, human data demonstrating that cerebrospinal fluid (CSF) levels of pregnenolone and dehydroepiandrosterone (DHEA) are correlated with temporal cortex levels of these respective neurosteroids within the same patient cohort are also consistent with proxy or surrogate biomarker potential for neurosteroid levels in more accessible tissues such as blood and CSF [45]. It should be noted, however, that several mechanisms for cerebral uptake of neurosteroids from peripheral blood circulation may influence central concentrations [46]. Given a compelling rationale informed by both preclinical and clinical findings from multiple research groups that implicate allopregnanolone dysregulation as a component in the pathophysiology of neurodegenerative disorders such as AD (and suggest a possible role for allopregnanolone or synthetic analogs in AD therapeutics), we thus investigated allopregnanolone levels in temporal cortex in patients with AD and cognitively intact control subjects. The overarching goal of the current study was to determine if we could replicate our prior neurosteroid findings in prefrontal cortex in male AD and male control patients in a second brain region (temporal cortex) utilizing samples from a larger cohort of subjects that includes both male and female patients.

In addition to allopregnanolone, other neurosteroids such as DHEA and pregnenolone may be candidate modulators of AD pathophysiology (see Fig. 1 for biosynthetic pathways). For example, DHEA appears to be elevated in postmortem brain tissue [38,47] and CSF [45,47,48] in AD patients compared to control subjects, and positively correlated with Braak and Braak neuropathological disease stage [38]. Like allopregnanolone, DHEA demonstrates a number of neuroprotective effects. For example, DHEA is protective against amyloid β-protein toxicity [49,50] and a number of other insults involving oxidative stress, including anoxia [51], glucocorticoid-induced toxicity [52,53], and NMDA-induced excitotoxicity [54]. In addition, DHEA enhances neurogenesis in rodent models and augments cell proliferation of human neural stem cells [52,55,56]. Pregnenolone may also play a role in the pathogenesis and clinical course of AD, given its neuroprotective effects against glutamate [57] and amyloid β-protein toxicity [58], and actions on learning and memory in animal models [59,60]. We therefore determined DHEA and pregnenolone levels in this postmortem brain tissue investigation using temporal cortex samples from patients with AD and cognitively intact control subjects. Since the current study includes temporal cortex postmortem tissue from males and females, this larger collection of samples will also provide data to determine if our prior neurosteroid findings in male subjects are also generalizable to female patients. To our knowledge, this is among the largest postmortem brain tissue investigations focusing on neurosteroids and AD to date.

In addition, this larger cohort of 40 patients with AD and 41 cognitively intact control subjects provides the opportunity to conduct exploratory analyses to determine if the presence of the APOE4 allele (the ε4 allele of apoliprotein E, or ApoE), a known risk factor for the development of late-onset AD [61], is associated with alterations in allopregnanolone, DHEA, and/or pregnenolone levels in temporal cortex. The mechanisms by which the APOE4 isoform of ApoE mediates AD risk are not yet completely understood. ApoE is a cholesterol transport protein that is present at high concentrations in the brain [62–64]. Since a major role of ApoE involves the regulation of cholesterol uptake into neurons (an action critical for synaptic function), and since cholesterol is the immediate precursor to pregnenolone (and pregnenolone is a precursor to other neurosteroids such as allopregnanolone), it is possible that AD risk conferred by the APOE4 allele may include a mechanism involving dysregulation in downstream events such as neurosteroid biosynthesis. To begin to test this possibility, we compared neurosteroid levels in temporal cortex in patients who are heterozygous or homozygous for the APOE4 allele, to subjects who do not carry this APOE isoform associated with elevated AD risk.

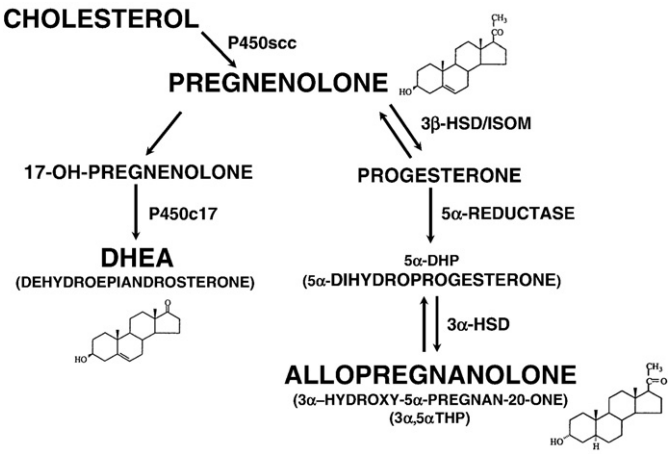

Fig. 1. Biosynthetic pathways and chemical structures for selected neurosteroids.

2. Materials and methods

2.1. Postmortem tissue

Frozen right hemisphere temporal cortex samples from a total of 81 patients were utilized in this investigation: samples from 40 subjects with AD (17 males, 23 females) and 41 cognitively intact control subjects (21 males, 20 females) from the Joseph and Kathleen Bryan Alzheimer's Disease Research Center (ADRC) collection at Duke University were analyzed for the neurosteroids allopregnanolone, DHEA, and pregnenolone by highly sensitive and specific gas chromatography/mass spectrometry (GC/MS) preceded by high performance liquid chromatography (HPLC) purification. A subset of this collection for which CSF was also available within the same cohort ($n=41$, approximately half of the total collection utilized for the current study) has been analyzed previously for pregnenolone and DHEA levels, and correlations of CSF pregnenolone and DHEA levels to respective temporal cortex levels of these two neurosteroids have been reported in an earlier investigation [45]. Allopregnanolone levels in temporal cortex and APOE findings have not been reported previously (except in poster format, [65]). Temporal lobe boundaries were the superior and middle temporal gyri. Subjects were enrolled in the ADRC autopsy and brain donation program, as described previously [66]. Procedures for enrollment were approved by the Duke University Medical Center Institutional Review Board. Cognitively intact control subjects had no neurological disorders. AD was diagnosed clinically according to National Institute of Neurological and Communicative Disorders/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria. AD diagnosis was confirmed at autopsy using the National Institute on Aging/Reagan Institute criteria. Neuropathological disease stage was determined