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Reduced CSF CART in dementia with Lewy bodies

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Abstract

Dementia with Lewy bodies (DLB) is the second most common form of neurodegenerative dementia after Alzheimer's disease (AD). The underlying neurobiological mechanism of DLB is not fully understood and no generally accepted biomarkers are yet available for the diagnosis of DLB. In a recent MRI study, DLB patients displayed hypothalamic atrophy whereas this region was not affected in AD patients. Cocaine and amphetamine regulated transcript (CART) is a neuropeptide expressed selectively in neurons in the hypothalamus. Here, we found that CSF CART levels were significantly reduced by 30% in DLB patients (n=12) compared to controls (n=12) as well as to AD patients (n=14) using radioimmunoassay. Our preliminary results suggest that reduced CSF CART is a sign of hypothalamic dysfunction in DLB and that it may serve as a biomarker for this patient group.

Keywords: dementia, biomarker, cocaine and amphetamine regulated transcript, Alzheimer's disease, cerebrospinal fluid

Introduction

Dementia is a common condition which affects 7% of the population older than 65 years and around 30% of individuals older than 80 years. Dementia can be defined as a syndrome of acquired impairment in several cognitive domains which in order to fulfil the criteria must be severe enough to cause impairment in social and occupational functioning [1]. There are numerous forms of dementia and many different underlying disorders. Alzheimer's disease (AD) is the most common form of dementia. Dementia with Lewy Bodies (DLB) is likely the second most common form of neurodegenerative dementia with a prevalence between 3% and 26% of all demented cases older than 65 years [9, 20]. The clinical picture of DLB constitutes of fluctuations in cognitive performance and consciousness, as well as Parkinsonism, visual hallucinations, falls and severe sensitivity to neuroleptic agents [9]. Accurate diagnosis of the different dementias as well as identification of specific biomarkers has important implications for current treatments and clinical trials for novel therapies. The diagnosis of AD has been improved since the identification of biomarkers indicative of AD in the cerebrospinal fluid (CSF) such as elevated levels of total tau (T-tau) and hyperphosphorylated tau (P-tau) together with reduced levels of β amyloid₁₋₄₂ (A β 42) [6]. No generally accepted biomarkers are yet available for the diagnosis of DLB. Emerging data suggest that imaging of the dopaminergic system will be important in differentiating DLB to AD, as loss of dopaminergic cells and dopamine transporters are a consistent finding in DLB [17].

In addition to the cognitive decline that is the central characteristic of dementia, depression and anxiety affect around 50% of all AD and DLB patients [8][3]. The underlying neurobiological mechanisms of psychiatric symptoms in DLB or AD are not fully understood. Altered levels of neuropeptides such as cocaine and amphetamine regulated transcript (CART) have been suggested to play a role in depression and anxiety [12]. CART is expressed in neurons in the hypothalamus that project to regions thought to regulate mood

such as the prefrontal cortex, the hippocampus and striatum [7] [14]. Mutations in the *CART* gene in humans are associated with depression and anxiety [5]. Interestingly, in a recent MRI study DLB patients displayed hypothalamic atrophy whereas this region was not affected in AD patients [18]. We therefore hypothesized that CART levels would be reduced in CSF from patients with DLB as a sign of hypothalamic pathology and compared these to levels in age-matched AD patients as well as healthy controls.

Materials and Methods

Subjects

CSF samples were obtained from 38 subjects at the Memory Disorders Clinic at Rigshospitalet in Copenhagen, Denmark, the Psychogeriatric Clinic at Lund University Hospital in Lund, and the Neuropsychiatric Clinic, Sahlgrenska University Hospital, Gothenburg, Sweden (Table 1). The study was approved by the medical ethics committees at the three hospitals. Patients fulfilled the consensus criteria for DLB [10] or AD (NINCDS-ADRDA) [11], respectively. Depression and anxiety were diagnosed according to the ICD-10 [19] and memory function was assessed with the mini mental state examination (MMSE). Controls were recruited from senior citizen organizations and via information meetings on dementia. Inclusion criteria were that the individuals should be in good physical and mental health and not experience or exhibit any cognitive impairment. Minor vascular or other disorders in a stable phase did not lead to exclusion. All controls were thoroughly interviewed about their somatic and mental health by a research nurse before inclusion in the study. In the AD group, seven patients were treated with anti-depressant medication, three with acetylcholine esterase inhibitor, one with anxiolytic medication, and two with neuroleptic medication, at the time of lumbar puncture. In the DLB group, six patients were treated with anti-depressant medication, four with acetylcholine esterase inhibitor, two with anxiolytic

medication, two with neuroleptic medication and five with L-dopa. No control individuals were treated with these medications. Measurements of T-tau, P-tau and A β 42 were performed as previously described using enzyme-linked immunosorbent assays (ELISA) [2][6].

CART measurement

CART levels were measured in CSF using a commercially available ^{125}I radioimmunoassay (RIA) kit (CART 55-102, Phoenix Pharmaceuticals, Belmont, CA, USA) [4]. Duplicate samples were assayed and levels were determined against a known standard. The inter-assay coefficients of variance (CV) is around 12%.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) program version 15.00 for Windows was used for all statistical analyses. For multiple comparisons, one-way and two-way Analyses of Variance (ANOVA) were used. For comparisons between two groups, Student's t-test was used. Correlation analyses were performed using Pearson's r.

Results

The study included CSF from 38 individuals (Table 1). No significant differences in age were found between the groups. All patients with AD showed typical changes for T-tau, P-tau and A β 42 in the CSF [6], with the A β 42/T-tau ratio < 1 in all cases. Patients with DLB showed either normal levels of CSF biomarkers or slightly increased T-tau and decreased A β 42, while P-tau levels were normal.

We detected a significant difference in CART levels between the three groups (one factor ANOVA, $p < 0.0001$) (Fig. 1). Post-hoc analyses revealed significant differences in CART between patients with DLB and controls (Scheffe's post-hoc test; $p < 0.0001$), patients with

DLB and AD (Scheffe's post hoc test; $p < 0.05$), and finally between patients with AD and controls (LSD post hoc test; $p < 0.05$). There were no significant differences in CART between women and men in the population as a whole or in the different diagnostic groups. Interestingly, CART levels correlated significantly with levels of P-tau (Pearson's $r = 0.37$, $p < 0.05$) and T-tau (Pearson's $r = 0.43$, $p < 0.01$) but not with A β 42 levels. However, CART levels in DLB or AD patients did not correlate with the severity of dementia assessed with MMSE. No significant differences in CART were found between patients with or without depression and anxiety in the population as a whole or in the different diagnostic groups (data not shown). There were also no differences in CART levels between AD or DLB patients with or without treatment with antidepressants, L-dopa or acetylcholine esterase inhibitor.

Discussion

We found significantly reduced levels of CART in CSF from DLB patients compared to AD patients and controls. CART is a neuropeptide expressed in neurons in the human hypothalamus. Hypothalamic atrophy has been found in DLB patients using voxel-based morphometry in MR images whereas no changes of this region was found in AD patients [18]. It is therefore possible that neurons producing CART in the hypothalamus are affected in DLB. Although Lewy bodies have been described in the hypothalamus of DLB patients [13], extensive neuropathological studies of this region in DLB have not yet been reported. Consequently, it would be of interest to study whether neurons expressing CART are affected in DLB as well as analyzing the role of reduced CSF-CART levels. Interestingly, intracerebroventricular injections of CART in rodents lead to increased dopaminergic activity in nucleus accumbens, striatum and hypothalamus [15]. Reduced CART levels may therefore be involved in causing or augmenting the dopaminergic hypofunction resulting in Parkinsonism as well as intolerance to antipsychotic agents in DLB [16]. CART has also been

suggested to play a role in depression and anxiety [12][14]. Although no significant differences in CART were found between patients with or without depression and anxiety in this small study population, further analysis of a larger cohort assessed using specific rating scales for psychiatric symptoms would be important for examining whether CART is involved in the psychiatric symptoms of DLB.

In conclusion, we found reduced levels of CART in patients with DLB compared to AD and healthy controls. A limitation of this study is the relatively small study population but we speculate that CSF CART could be used as an additional marker for differential diagnosis between DLB and AD. In addition, our findings might contribute to the understanding of pathophysiological differences between these two diseases.

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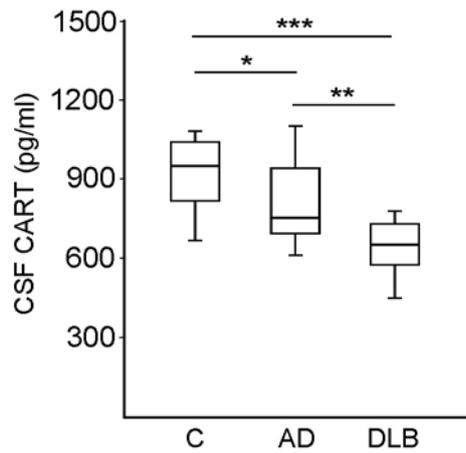


Figure legend

Figure 1. The level of CSF CART was significantly lower in DLB patients (n=12; mean =642, CV: 0.16) compared to AD patients (n=14; mean =794, CV: 0.18) and controls (n=12; mean =917, CV: 0.15) (one factor ANOVA, $p < 0.0001$; *** = $p < 0.0001$, Scheffe's post-hoc test, ** = $p < 0.05$, Scheffe's post hoc test, and * = $p < 0.05$, LSD post hoc test).

Comparisons between controls and subjects with Alzheimer's disease and dementia with Lewy bodies

Parameters	Controls	AD	DLB	Significant difference
Gender (female/male)	8/4	11/3	5/7	not applicable
Age (years)	70 ± 1.6	72 ± 3.2	73 ± 4.1	n.s.
MMSE (points)	29.4 ± 0.9	21.9 ± 3.1	22.3 ± 4.3	a, b,
Aβ42 (pg/ml)	718 ± 288 (0.40)	303 ± 104 (0.34)	539 ± 215 (0.40)	a
T-tau (pg/ml)	480 ± 161 (0.34)	618 ± 342 (0.55)	328 ± 131 (0.40)	e
P-tau (pg/ml)	76 ± 30 (0.40)	143 ± 91 (0.64)	44 ± 17 (0.39)	d, c

The data is expressed as the mean value ± SD (CV). Significant difference between the groups, as measured with Scheffe's post hoc test: AD and controls, p <0.01 (a); DLB and controls, p <0.01 (b); AD and DLB, p <0.01 (c); AD and controls, p <0.05 (d); AD and DLB, p <0.05 (e).

Dementia with Lewy bodies (DLB), Alzheimer's disease (AD), mini mental state examination (MMSE).