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**Cocaine and amphetamine regulated transcript is increased
in Huntington disease**

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Abstract

Weight loss and anxiety frequently occur in Huntington's disease (HD) but the underlying mechanisms are not well understood. Peptides produced in the hypothalamus are involved in regulating energy homeostasis and emotion. Recent data suggest that changes in neuropeptide levels may be reflected in the cerebrospinal fluid (CSF), and could therefore serve as biomarkers in HD. Cocaine- and amphetamine regulated transcript (CART) is a neuropeptide expressed in several brain regions such as the hypothalamus, amygdala, and hippocampus. CART has been shown to increase anxiety and reduce food intake in rodents by as yet unknown mechanisms. Individuals with a *CART* mutation exhibit increased anxiety. In cross-sectional CSF samples from HD patients (n=39), we found that levels of CART peptide were significantly increased by 23% compared to control subjects (n=28). Increased CART levels in HD therefore holds promise as a biomarker as well as a potential pathogenic mediator of symptoms.

Key words: anxiety, hypothalamus, CART, biomarker, CSF

Introduction

Huntington's disease (HD) is a CAG triplet repeat disorder with psychiatric, motor, and cognitive manifestations. Classically, it is characterized by atrophy and cell death in striatum and cerebral cortex, but pathology has also been observed in the hypothalamus (1-4). The disease-causing gene was discovered in 1993 (5) and is an important diagnostic tool. It is one of the few identified "trait biomarkers" in neurology of today. In contrast, reliable and objective biomarkers to determine the stage and progression of the disease in HD are still missing (i.e. state biomarkers; 6). Emerging data suggest that important neuroendocrine changes occur in HD (7). These changes could be involved in causing weight loss (8) as well as psychiatric symptoms, such as anxiety (9) in HD. Neuroendocrine factors are released into the cerebrospinal fluid (CSF), blood and urine and their levels may provide appropriate biomarkers for HD. In this study we have determined the level of cocaine and amphetamine regulated transcript (CART) in the CSF of HD patients and control subjects. CART is produced in several brain regions such as the hypothalamus, the amygdala and the hippocampus (10), areas involved in regulation of energy homeostasis, stress and anxiety (11). Increased levels of CART leads to increased anxiety (11) and reduced food-intake (12) in rodents. Altered levels of CART may be associated with some of the clinical manifestations of HD. We therefore determined CART levels in the CSF of HD patients.

Methods

We collected CSF (1-2 ml) from well-characterized early to mid-stage HD patients (n = 39, average age 53 ± 2 years; females: 10, males: 29) and healthy control subjects (n =

28, average age 49 ± 2 years; females: 14, males: 14) in the clinics of Dr. Jørgen Nielsen (University of Copenhagen, Denmark) and Drs Bernhard Landwehrmeyer/Daniel Ecker (Ulm University, Germany) within the Euro-HD Network, and from Dr. Blair Leavitt (University of British Columbia, Canada) within the High Q Biomarker Consortium. The experiment was approved by the institutional review board at the respective universities and informed consent was obtained from the subjects.

Results

We measured levels of CART using a commercially available ^{125}I RIA kit (Phoenix Pharmaceuticals, Belmont, CA, USA). Duplicate samples were assayed and levels were determined against a known standard. We found that CART levels were 111 ± 6 pmol/l (mean \pm SEM) in control subjects (n=28) compared to 144 ± 5 pmol/l (n=39) in HD patients ($P < 0.0001$, two-tailed unpaired Students t-test) (Fig. 1). The mean CAG repeat size in the HD group was 44 ± 1 CAG repeats. Linear regression analysis did not reveal any correlation between CAG repeat length and CART levels in the HD patients (R square 0.10, $p = 0.06$). Data on body mass index (BMI) were available for 36 of the HD patients in this study and the mean was 25.2 ± 0.8 (kg/m²). Linear regression analysis revealed no correlation between BMI and CART levels in the HD patients (R square – 0.007, $p = 0.62$). Anxiety was present in 18 out of 37 HD patients (data not available for 2 HD patients). No difference was found in CART levels between the group of HD patients with anxiety (147 ± 6 pmol/l) versus the group without anxiety (135 ± 6 pmol/l) (Student's t-test, n.s.). Data on medication were available for 28 of the HD patients. Selective serotonin/noradrenalin reuptake inhibitors (SSRI/SNRI) are used to treat

depression and anxiety, and were taken by 15 out of the 28 HD patients. No significant difference was found in CART levels between HD patients that were on SSRI/SNRI treatment (142.4 ± 6.9 pmol/l) compared to non-treated HD patients (136.9 ± 8.2 pmol/l; Students t-test, n.s.)

Discussion

In this study, we have shown that levels of CART were increased in CSF of HD patients compared to control subjects and may constitute a novel biomarker for HD. While blood would be a more accessible compartment for a biomarker, CART in blood is thought to be mainly derived from the adrenal gland (13). Moreover, CART is difficult to measure in blood. The presence of CART has only been possible to demonstrate in blood after extensive extraction in a few studies and never in human samples (13). Our findings of increased CART levels in the CSF of HD patients are interesting, but further studies in a larger cohort with longitudinal CSF samples are required. It is important to determine at what disease stage CART levels are altered and if the change progresses over time. Changes in CART in HD may underlie some of the clinical symptoms. Experiments mainly in rodents have shown that CART is a strong anorectic peptide involved in regulating satiety and body weight (12) and several reports have demonstrated its role in drug addiction (14). Administration of CART in animals leads to increased anxiety (11). It may mediate anxiety through the endogenous cannabinoid system or via corticotropin releasing factor and the hypothalamic-pituitary-adrenal axis (11). A recent study has identified a mutation in the *CART* gene that results in obesity and increased anxiety in affected members of an Italian family (15). In HD, psychiatric symptoms often occur at

an early stage and include increased anxiety levels. The pathological substrate for anxiety in HD is not known. We hypothesized that increased CART levels could be involved in causing anxiety in HD. In this study however, we found no significant difference in CART levels between subgroups of our HD patients with and without anxiety. Weight loss is also an early sign of the disease (8) and increased CART levels may play a role in altering the energy homeostasis in HD. The HD patients in this study had a mean BMI of 25.2 ± 0.8 (kg/m²) and were of normal weight. Information on whether they had lost weight during the disease process is not available. It is therefore not possible to determine whether the increase in CART levels is primary or secondary to weight changes. It is possible that the increase in CART levels is due to reduced expression levels or altered affinity of CART receptors. We did not detect any correlation between BMI and CART levels in the HD patients. Weight loss is a multifactorial process that could be related to increased energy expenditure, dysphagia, disrupted eating behavior and psychosocial factors limiting optimal nutrition. Interestingly, in HD, weight loss occurs despite adequate nutrition (16) and carbohydrate-enriched diets (17, 18), which could not be explained by increased CART levels. Whether CART play a role in any other aspects of the weight loss warrants further investigation.

In conclusion, CSF-CART is a potential biomarker for HD. Future studies will determine whether increased CART levels are involved in mediating symptoms in HD. .

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Figure legend

Fig 1.

CART levels in CSF from HD patients (HD; n = 39) are increased by 23% compared to controls (C; n = 28) (mean \pm SEM; Students t-test $P < 0.0001$).

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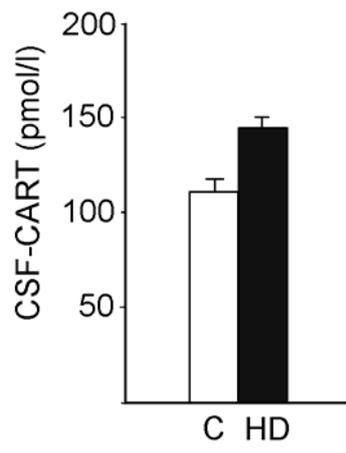


Figure 1