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Relationship between cerebrospinal fluid concentrations of orexin A/hypocretin-1 and body composition in humans

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ABSTRACT

The hypothalamic neuropeptide orexin A (hypocretin-1) is a key signal in sleep/wake regulation and promotes food intake. We investigated the relationship between cerebrospinal fluid orexin A concentrations and body composition in non-narcoleptic human subjects with a wide range of body weight to gain insight into the role of orexin A in human metabolism. We collected cerebrospinal fluid and blood samples and measured body composition by bioelectric impedance analysis in 36 subjects (16 women and 20 men) with body mass indices between 16.24 and 38.10 kg/m^2 and an age range of 19–80 years. Bivariate Pearson correlations and stepwise multiple regressions were calculated to determine associations between orexin A and body composition as well as biometric variables. Concentrations of orexin A in cerebrospinal fluid averaged 315.6 ± 6.0 pg/ml, were comparable between sexes ($p > 0.15$) and unrelated to age ($p > 0.66$); they appeared slightly reduced in overweight/obese compared to normal-weight subjects ($p = .07$). Orexin A concentrations decreased with body weight ($r = -0.38$, $p = .0229$) and fat-free mass ($r = -0.39$, $p = .0173$) but were not linked to body fat mass ($p > 0.24$). They were inversely related to total body water ($r = -0.39$, $p = .0174$) as well as intracellular ($r = -0.41$, $p = .0139$) and extracellular water ($r = -0.35$, $p = .0341$). Intracellular water was the only factor independently associated with cerebrospinal fluid orexin A concentrations ($p = .0139$). We conclude that cerebrospinal fluid orexin A concentrations do not display associations with body adiposity, but are inversely related to intracellular water content. These cross-sectional findings suggest a link between orexin A signaling and the regulation of water homeostasis in humans.

1. Introduction

The neuropeptide orexin A (hypocretin-1) is mainly expressed by neurons in the lateral hypothalamus; it promotes wakefulness and stabilizes arousal [1,2], while also displaying orexigenic properties [3,4]. Orexinergic neurons connect to a broad network of central nervous regions including the hypothalamic arcuate nucleus, a central hub of metabolic control where peptidergic messengers such as proopiomelanocortin and neuropeptide Y interact to regulate feeding behavior [5]. Orexin A stimulates food anticipatory behavior and food intake, especially with regard to reward-driven eating [6,7]. The abundance of glucose sensors and receptors for leptin and ghrelin in orexinergic neurons [8] further indicates that orexin is essential for adapting the level of alertness to metabolic needs [9]. Vice versa, impairments in orexinergic signaling might help explain why insufficient and impaired sleep predisposes to increases in food intake [10] and elevated body weight (11; for review see reference 12). The contribution of orexin A to metabolic function extends to glucose homeostasis: high doses of orexin A administered to rats promote hepatic glucose release and increases in blood glucose [13,14]. Respective studies in mice indicate that the peptide bidirectionally fine-tunes hepatic gluconeogenesis by regulating autonomic balance, and point towards a role of the orexin

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system in the regulation of circadian blood glucose oscillations [15].

Adipose tissue interacts with orexin A in metabolic control [16]. Leptin and ghrelin inhibit and, respectively, enhance orexin A signaling in the brain [17,18]. Orexin knock-out (KO) in comparison to wild-type (WT) mice display sex-specific body weight and body composition changes [19,20]. Adult and aged female orexin KO mice have elevated body weight, a higher proportion of fat, muscle and free fluid, whereas male orexin KO mice do not differ from WT counterparts in body weight but carry more body fat [20]. By affecting the accumulation of brown adipose tissue, orexin A indirectly determines metabolic rate and thermogenesis [2,21]. In rats, intracerebroventricular administration of high doses of orexin A induces lipolysis via histamine receptor-mediated effects on sympathetic activation, whereas low doses have opposing effects [22].

Most insights into the function of orexin A derive from animal studies, while the lion's share of what is known about orexin A signaling and body weight regulation in humans concerns specific clinical aspects. Orexin A is deficient in patients with narcolepsy with cataplexy [23], a neurological disorder characterized by impaired sleep/wake regulation leading to excessive daytime sleepiness, and sudden episodes of partial or total loss of muscle tone. These patients, and also respective animal models of orexin A deficiency [19,24], show a tendency towards overweight [25], even though food-seeking behavior appears to be attenuated [26,27]. Interestingly, circulating leptin levels in narcolepsy have been reported to be comparable to [28] or lower [29,30] than those of healthy controls. In order to investigate the interplay between central nervous orexin A signaling and body weight regulation, we investigated the relationship between body composition and cerebrospinal fluid (CSF) concentrations of orexin A in a sample of non-narcoleptic participants with a wide range of body-mass index (BMI). Considering the stimulatory role of orexin A in food intake control, we expected to find indicators linking CSF concentrations of the peptide to body fat content.

2. Methods

2.1. Participants

Forty-one Caucasian subjects (21 men and 20 women) aged between 19 and 80 years (mean age ± SEM, 52.98 ± 2.29 years) were included in the study. They had a BMI range of 16.24–38.10 kg/m^2 and a mean BMI ± SEM of 26.68 ± 0.70 kg/m^2. Fifteen subjects were normal-weight and 26 subjects were overweight or obese (BMI ≥ 25). Exclusion criteria were an anamnestic history of diabetes, congestive heart failure, liver or kidney disease, malignancy, signs of inflammation, pregnancy, and any drugs influencing body weight like corticoids, diuretics or contraceptives. Five subjects (one woman and four men) were newly diagnosed with type 2 diabetes due to fasting plasma glucose levels > 7 mmol/l according to the criteria of the American Diabetes Association. Since their exclusion did not essentially alter the results, their data were included in the analyses. Three subjects were excluded from analyses because of CSF orexin A concentrations below 110 pg/ml, which are indicative of orexin A deficiency [31], and two further subjects because of outlying values below the group average minus two standard deviations. None of these or the remaining subjects reported narcoleptic symptoms (e.g., sleep disturbances, excessive daytime sleepiness, catalepsy), or worked in shifts. All participants gave written informed consent to the study that conformed to the Declaration of Helsinki and was approved by the local ethics committee.

2.2. Collection of blood and CSF samples and assessment of body composition

After an overnight fast with caffeine restriction but unlimited water supply, subjects reported to the lab in the morning between 07:00 and 09:00 for simultaneous sampling of blood and CSF (1 ml) via lumbar puncture after local anesthesia (2 ml mepivacain-HCl 1%). Blood samples were immediately centrifuged, and plasma and CSF samples were frozen at −80 °C until assay. We assessed BMI and waist-to-hip ratio and measured body composition by standard multifrequency bioelectric impedance analysis (BIA; BIA 2000-M, Data Input GmbH, Frankfurt, Germany). Frequencies of 1, 5, 50 and 100 Hz were employed and results analyzed with Eurobody software (Data Input GmbH, Frankfurt, Germany). This safe and non-invasive technique estimates total body water (TBW), extracellular water (ECW), intracellular water (ICW), fat mass (FM) and fat-free mass (FFM) using equations validated for different populations (for in-depth information see reference 32).

CSF concentrations of orexin A were measured by means of a commercially available [^{125}I] radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA, USA) with a detection limit of 50 pg/ml and an intra-assay variability below 10%. All samples were assayed in duplicate and measured in the same kit at the same time. Measurements were evaluated using a standard curve and concentrations of CSF orexin A were determined against a set of four internal standard CSF samples (see reference 33 for further details). In addition, plasma and CSF glucose concentrations were measured (Beckman Glucose Analyzer II; Beckman Instruments, Munich, Germany). Insulin was determined using a commercial competitive double-antibody RIA (Pharmacia Insulin RIA 100; Pharmacia Diagnostics, Upsalla, Sweden). Assay sensitivity was increased to a threshold of 1.8 pmol/l by using 100 µl of CSF, 50 µl of [^{125}I] insulin diluted with buffer at a ratio of 1:3, and 50 µl of insulin antiserum diluted at a ratio of 1:2 (incubation time of 3 h; intra-assay variation was < 4.5%). Adiponectin concentrations in plasma and CSF were determined using a commercially available radioimmunoassay kit (Linco Research, St. Charles, MO) according to the manufacturer's protocol, with an intraassay coefficient of variation of 6.2%.

2.3. Statistical analyses

Data of 36 subjects (four and ten normal-weight, 16 and six overweight or obese men and, respectively, women) entered analyses (Table 1). Bivariate Pearson correlations and stepwise multiple regressions were calculated to determine associations between CSF orexin A concentrations and relevant variables (age, body weight, waist and hip circumference, BMI, fat mass, total body water, intracellular water, extracellular water, body cell mass and CSF and plasma concentrations of glucose, insulin and adiponectin). Two-tailed *t*-tests were used to compare differences in CSF orexin concentrations between different groups (male/female, lean/obese); interactions between sex and body weight status were analyzed by ANOVA. We conducted statistical

Table 1
Subject characteristics and correlations with CSF orexin A concentrations.

	Mean (SEM)	Correlation with CSF orexin A (95% CI)	*p* value
Age (years)	53.4 (2.70)	−0.07 (-0.39, 0.26)	0.667
BMI (kg/m^2)	26.6 (0.81)	−0.23 (-0.52, 0.10)	0.172
Body weight (kg)	78.0 (3.0)	−0.38 (-0.63, −0.06)	0.023
Body fat mass (kg)	21.8 (1.77)	−0.20 (-0.50, 0.14)	0.244
Fat-free mass (kg)	56.2 (1.98)	−0.40 (-0.64, −0.08)	0.017
Body cell mass (kg)	29.0 (1.22)	−0.27 (-0.55, 0.07)	0.113
Total body water (l)	41.1 (1.45)	−0.39 (-0.64, −0.08)	0.017
Intracellular water (l)	24.4 (0.95)	−0.41 (-0.65, −0.09)	0.014
Extracellular water (l)	16.8 (0.52)	−0.35 (-0.61, −0.03)	0.034
Plasma glucose (mmol/l)	5.4 (0.19)	0.28 (−0.05, 0.56)	0.096
CSF glucose (mmol/l)	3.38 (0.08)	0.04 (−0.29, 0.36)	0.815
Plasma insulin (pmol/l)	81.1 (11.33)	−0.07 (-0.39, 0.26)	0.681
CSF insulin (pmol/l)	2.9 (0.21)	0.12 (−0.22, 0.43)	0.493
Plasma adiponectin (ng/ml)	12225 (867.35)	0.17 (−0.17, 0.47)	0.321
CSF adiponectin (ng/ml)	6.25 (1.25)	−0.05 (-0.37, 0.29)	0.788
Waist circumference (cm)	95.3 (2.60)	−0.36 (-0.62, −0.03)	0.036