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Central and Peripheral Endocannabinoids and Cognate Acylethanolamides in Humans: Association with Race, Adiposity, and Energy Expenditure

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Context: Peripheral and central endocannabinoids and cognate acylethanolamides (AEs) may play important but distinct roles in regulating energy balance.

Objective: We hypothesized that in humans central/peripheral endocannabinoids are differently associated with adiposity and energy expenditure and differ by race.

Design: We examined associations of arachindonoylethanolamide, 2-arachidonoylglycerol, palmitoylethanolamide, and oleoylethanolamide (OEA) assayed in plasma and cerebrospinal fluid (CSF) with race, adiposity, and energy expenditure.

Setting/Participants: In this monitored clinical inpatient study, CSF was obtained by lumbar puncture in 27 individuals (12 Caucasian, 11 American Indian, and four African-American). Twenty-four hour and sleep energy expenditure were measured by indirect calorimetry in a respiratory chamber.

Main Outcome Measure: Samples were analyzed from a previous study originally designed to test a blood-brain barrier leptin transport deficit in human obesity.

Results: CSF (but not peripheral) 2-arachidonoylglycerol was significantly increased in American Indians compared with Caucasians (18.48 ± 6.17 *vs.* 10.62 ± 4.58 pmol/ml, $P < 0.01$). In the whole group, peripheral AEs were positively but in CSF negatively associated with adiposity. However, in multivariate models adjusted for the other peripheral and CSF AEs, peripheral arachindonoylethanolamide was the only AE significantly associated with adiposity. Interestingly, CSF OEA concentrations were positively associated with adjusted 24 hour and sleep energy expenditure ($r = 0.47$, $P < 0.05$; $r = 0.42$, $P < 0.05$), but peripheral OEA was not.

Conclusions: These data indicate a central alteration of the endocannabinoid system in American Indians and furthermore show that AEs in both compartments play an important but distinct role in human energy balance regulation. **(*J Clin Endocrinol Metab* 96: 787–791, 2011)**

The endogenous cannabinoid (CB) system modulates energy balance and thus may be etiologic in human obesity (1). Arachidonoylethanol (AEA) and specifically 2-arachidonoylglycerol (2-AG) increase during fasting and decline during food ingestion in the rat hypothalamus and limbic forebrain, regions that are highly involved in appetite and body weight regulation (2). In animal experiments, oral administration of CB receptor antagonists

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Abbreviations: AE, Acylethanolamide; AEA, arachidonoylethanol; 2-AG, 2-arachidonoylglycerol; CB, cannabinoid; CNS, central nervous system; CSF, cerebrospinal fluid; EC, endocannabinoid; EE, energy expenditure; 24EE, 24-h EE; OEA, oleoylethanolamide; OGTT, oral glucose tolerance test; PEA, palmitoylethanolamide; PPAR, peroxisomal proliferator-activated receptor; SLEEP, sleep EE.

reduced both food intake and body weight, probably via blockage of central CB-1 receptors in the hypothalamus (3). In addition to the central effects of endocannabinoids (ECs), recent research provides increasing evidence of peripheral dysregulation of ECs and related acylethanolamides (AEs) in obesity and type 2 diabetes (4). For example, nonobese healthy individuals exhibit reduced oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) levels in the circulation after ingestion of a meal. In healthy lean compared with weight-matched diabetic individuals, OEA and PEA are elevated (5). Furthermore, AEA, OEA, and PEA concentrations measured in sc fat are elevated and positively correlated with each other in obese diabetic individuals (6). Recently, Izzo *et al.* have demonstrated in rats that PEA and OEA concentrations vary acutely after food deprivation and refeeding in various tissues (liver, pancreas, duodenum, sc, and visceral fat) (7). Also, increased plasma AEA and 2-AG levels have been reported in obese *vs.* lean menopausal women (8), and elevated fasting levels of 2-AG but not AEA have been observed in men with increased visceral obesity (9). OEA and PEA, which are structurally similar to CBs, exert their effects via peroxisomal proliferator-activated receptor (PPAR)-α, vanilloid, and G protein-coupled receptors (TRPV-1, G protein-coupled receptor-119) (10, 11). Furthermore, peripheral OEA has recently been linked to perturbations of circadian rhythm, a risk factor for the development of metabolic diseases (12).

Novel selective peripheral CB1 antagonists in mouse experiments have recently shown beneficial effects on body weight, glucose, and lipid metabolism but sparing behavioral changes seen with the central nervous system (CNS) penetrating CB1 antagonist rimonabant (13). These results indicate a need for further characterization of the human EC system in the periphery and simultaneously the CNS. Therefore, we measured AEA, 2-AG, OEA, and PEA in plasma and cerebrospinal fluid (CSF) from 27 individuals with diverse racial background and various measures of adiposity and energy expenditure.

Materials and Methods

Study outline

Nonsmoking healthy volunteers (n = 27) were admitted to our clinical research unit receiving a weight-maintaining diet (50% carbohydrate, 30% fat, 20% protein). Dual-energy x-ray absorptiometry and a 75-g oral glucose test were used to assess anthropometry and glucose metabolism. On d 3, 24-h and sleep energy expenditure (24EE, SLEEP) were measured in a metabolic chamber as described elsewhere (14). Thereafter volunteers underwent lumbar puncture for collection of 8 ml CSF. All subjects provided written and informed consent before study participation. The protocol and consent form were approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Disease.

Glucose, insulin, leptin, and EC/AE measurements

Plasma glucose concentrations were determined by the glucose oxidase method and insulin concentrations were measured by Concept 4 RIA (ICN Pharmaceutical Inc., Costa Mesa, CA). Leptin concentrations were measured with a solid-phase sandwich enzyme immunoassay. Plasma and CSF levels of AEA, OEA, PEA, and 2-AG were quantified by liquid chromatography mass spectrometry.

Statistical analyses

Statistical analyses were performed using SAS Enterprise guide 9.1 (SAS Institute, Cary, NC). Mean values of multiple variables were compared using ANOVA with *post hoc* Tukey-Kramer tests. Distribution of variables was tested by evaluation of histograms and probability plots. Pearson (for normally distributed variables) or Spearman (for skewed variables) correlation tests were used to test associations of EC/acylethanolamide concentrations with measures of adiposity adjusted for age and sex. To adjust energy expenditure (EE) for age, sex, and body size, variables of EE were pooled with previously measured energy expenditure data from 1175 nondiabetic individuals from a longitudinal study of diabetes and obesity (EE group) and adjusted for age, gender, fat mass, fat-free mass, and race with an additional adjustment for physical activity for 24EE. Residuals were then extracted and used to perform correlation analyses with EC/acylethanolamide variables. Both groups were similar in age and adiposity as depicted in Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org. However, the EE group had slightly higher fasting glucose values compared with the current study group, which led us to further adjust for fasting glucose in these multivariate regression models. The alpha was set at 0.05.

Results

Subject characteristics are shown in Supplemental Table 1. As expected, EC/AE plasma concentrations were significantly higher than CSF concentrations (AEA: 1.12 ± 0.53 *vs.* 0.06 ± 0.08 pmol/ml; 2-AG: 37.62 ± 30.74 *vs.* 14.20 ± 6.85, PEA: 7.63 ± 1.91 *vs.* 2.12 ± 0.72; OEA: 6.40 ± 1.69 *vs.* 0.91 ± 0.35; for all $P < 0.001$). In plasma, AEA, 2-AG, PEA, and OEA and in CSF AEA, PEA and OEA concentrations did not differ by race; however, 2-AG concentrations were higher in CSF of American Indian individuals compared with Caucasians ($P < 0.01$) and differed significantly across groups (see Fig. 1 and Supplemental Table 1).

Associations of central and peripheral ECs/AEs with different measures of adiposity are presented in Table 1. Peripheral AEA concentrations were strongly positively associated with all available measures of adiposity. In contrast, CSF, AEA, and PEA were negatively associated with

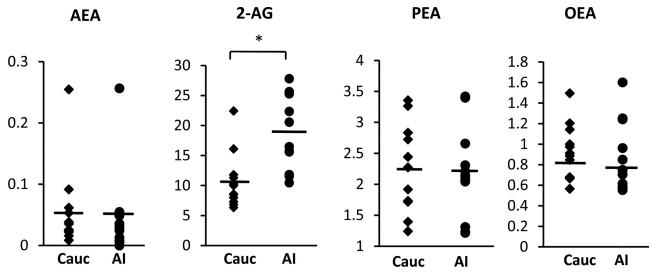

FIG. 1. EC and AE concentrations in CSF of Caucasian (Cauc) and American Indian (AI) individuals. Distribution and comparison of AEA, 2-AG, PEA, and OEA measurements (picomoles per milliliter) in CSF of Caucasian and American Indian individuals are shown. *Horizontal bars* represent group means. Due to the low sample size of African-American individuals (n = 4), this plot is depicting only EC and AE differences in CSF between Caucasians and American Indians. *, $P = 0.002$.

measures of adiposity. Furthermore, peripheral PEA and OEA as well correlated positively with adiposity. In multivariate models adjusting for peripheral AEA concentrations, these associations became nonsignificant. Additionally, we found that peripheral AEA levels (but not other peripheral or central ECs/AEs) were positively associated with fasting insulin levels and incremental insulin area under curve during an oral glucose tolerance test (OGTT) (r = 0.50, $P = 0.02$; r = 0.50, $P = 0.03$), two measures of insulin resistance; however, these associations were no longer significant after accounting for adiposity (F = 2.72, $P = 0.12$; F = 0.18, $P = 0.67$).

Acylethanolamides were significantly correlated with each other in cerebrospinal fluid and plasma, respectively; however, there were no associations with 2-AG in either of the two compartments (Table 1). Furthermore, we measured leptin concentrations in plasma and CSF. As expected, leptin was strongly associated with percent body fat (CSF: r = 0.869, $P < 0.0001$; plasma: r = 0.908, $P < 0.0001$) and all other measures of adiposity in both compartments (data not shown). However, no associations between leptin and ECs or AEs were observed (data not shown).

Finally, neither central nor peripheral ECs were associated with 24EE [AEA: $r = 0.27$ (CSF), 0.37 (plasma); 2-AG: 0.06, 0.18] or SLEEP (AEA: 0.41, −0.09; 2-AG: 0.14, −0.05). Also, PEA in both compartments was not associated with 24EE (0.33, −0.25) or SLEEP (0.07, −0.13). However, OEA in CSF (but not plasma, $r = -0.18$) was strongly positively associated with both 24EE and SLEEP (0.47, $P < 0.05$ and 0.42, $P < 0.05$) as shown in Supplemental Fig. 1.

Discussion

The comparison of EC/AE concentrations in plasma and CSF between ethnic groups revealed that American Indians have almost 2-fold increased 2-AG concentrations in CSF compared with Caucasians. This dysregulation of the central EC system could contribute to their increased propensity to develop obesity and diabetes (15).

In the whole study population, AEA concentrations in plasma were strongly positively associated with different measures of adiposity as shown in Table 1. Other studies have also shown higher concentrations of circulating AEA in individuals with increased adiposity (8, 16). Although previous mouse experiments have provided evidence that central administration of leptin reduces EC tone (17), we did not observe any association between CSF AEA and

TABLE 1. Correlations of central and peripheral ECs and AEs

Variable	Mean	AEA Pl	2-AG Pl	PEA Pl	OEA Pl	AEA CSF	2-AG CSF	PEA CSF	OEA CSF
AEA Pl	1.12 ± 0.53	1	—	—	—	—	—	—	—
2-AG Pl	37.62 ± 30.74	*−0.08*	1	—	—	—	—	—	—
PEA Pl	7.63 ± 1.91	*0.73*[a]	*−0.17*	1	—	—	—	—	—
OEA Pl	6.40 ± 1.69	*0.66*[b]	*−0.04*	*0.71*[a]	1	—	—	—	—
AEA CSF	0.06 ± 0.08	*−0.24*	*0.10*	*−0.18*	−0.19	1	—	—	—
2-AG CSF	14.20 ± 6.85	*−0.16*	*0.12*	*−0.20*	0.12	*0.34*[c]	1	—	—
PEA CSF	2.12 ± 0.72	*−0.25*	*−0.03*	*−0.19*	−0.24	*0.60*[d]	0.22	1	—
OEA CSF	0.91 ± 0.35	*−0.20*	*0.11*	*−0.23*	−0.20	*0.78*[a]	0.37[c]	0.48[e]	1
BMI	33.71 ± 8.45	*0.67*[b]	*−0.15*	*0.51*[d,f]	0.42[e,f]	*−0.56*[d,f]	−0.27	−0.40[e,f]	*−0.24*
PFAT	31.40 ± 10.48	*0.51*[d]	*−0.26*	*0.4*[e,f]	0.38[c]	*−0.42*[e,f]	−0.10	−0.35[c]	*−0.11*
Weight	96.67 ± 25.37	*0.69*[a]	*−0.05*	*0.51*[d,f]	0.42[e,f]	*−0.44*[e,f]	−0.31	−0.35[c]	*−0.19*
Fat mass	31.99 ± 15.85	*0.68*[a]	*−0.14*	*0.53*[d,f]	0.43[e,f]	*−0.57*[d,f]	−0.28	−0.44[e,f]	*−0.23*
Waist	108.2 ± 19.1	*0.69*[a]	*−0.14*	*0.48*[e,f]	0.44[e,f]	*−0.47*[e,f]	−0.23	−0.31	*−0.13*

Values are presented as mean ± SD. *R* values represent either Pearson or Spearman correlation coefficients between correlation pairs. Pearson correlation coefficient was used for Gaussian-distributed variables and Spearman for skewed variables. Spearman correlation coefficients are *italicized*. All associations with measures of adiposity are adjusted for age and sex. BMI, Body mass index (kilograms per square meter); PFAT, body fat percentage; weight, body weight (kilograms); fat mass, total body fat mass (kilograms); waist, waist circumference (inches); Pl, measured in plasma. Dashes represent redundant correlations.

[a] $P < 0.0001$; [b] $P < 0.001$; [c] $P = 0.05$–0.10; [d] $P < 0.01$; [e] $P < 0.05$; [f] associations were no longer significant after adjustment for peripheral AEA concentrations.

leptin (data not shown). However, the reductions in AEA and 2-AG by leptin were observed only in lean rats and obese leptin-sensitive (*ob/ob*) mice. In our study, the lack of association of leptin with ECs could be due to relative leptin insensitivity in our obese individuals. In support of this, there was a negative association of AEA with leptin in lean healthy or anorectic women but not in obese women with binge-eating disorder (16). Furthermore, peripheral AEA levels were positively associated with fasting insulin levels and incremental insulin area under curve during an OGTT, two measures of insulin resistance. These results are consistent with data published by Di Marzo *et al.* (18) showing that circulating AEA concentrations are decreased during an OGTT and euglycemic insulin clamp experiments proportional to the changes in insulin action.

In multivariate models including plasma and CSF AEA, plasma AEA levels remained strongly related to adiposity, whereas CSF AEA did not, implicating a strong peripheral dysregulation of AEA in human adiposity. This is supportive of recently published data by Tam *et al.* (13) showing that a selective peripheral CB1antagonist can potently induce weight loss and exert beneficial effects on glucose and lipid metabolism in mice without the central side effects observed using rimonabant, a CNS-penetrating CB1 receptor antagonist. Furthermore, adjustment of plasma PEA and OEA for peripheral AEA abrogated the associations with adiposity measures. This is not surprising because all three compounds are products of the same biosynthetic pathway via release of *n*-acyl-phosphatidylethanolamides mediated by phospholipase-D as previously described (19). However, this conclusion has to be drawn cautiously because OEA levels in peripheral organs of obese rats can vary substantially, and inactivation of these acylethanolamides can be accomplished to a varied extent by different amidases (fatty acid amide hydrolase 1/2, n-acylethanolamine-hydrolyzing acid amidase) (7, 20).

Most interestingly, OEA concentrations in CSF were positively associated with different measures of EE (Supplemental Fig. 1). Experiments in rodents indicate a role for OEA in regulating energy metabolism by enhancing lipolysis (11). However, we did not observe associations between CSF OEA and lipid oxidation. The OEA receptor PPAR-α has recently been shown in neurons of the nuclei of solitary tract in the brain stem (7), a region involved in the modulation of energy balance. Therefore, central activation of PPAR-α via OEA could explain the positive association with EE traits.

We must acknowledge that the sample size for this analysis was limited, but this group is unique in that we analyzed paired CSF and plasma samples in individuals with detailed metabolic characterization. Furthermore, circulating concentrations of ECs/AEs are thought to reflect *ad hoc* activation of synthesizing enzymes in the cell and subsequent release into the circulation (organ spillover) due to their lipophilic nature. Assumptions on the activation status of specific peripheral organs cannot be made unless specific organ tissue samples are analyzed. In the brain, EC and AE target receptors are expressed on postsynaptic membranes, and secretion of these neuronal signal molecules into the neuronal gap could increase concentrations in the interstitium and also the CSF. Whether concentrations of ECs and AEs in the CSF simply reflect increased neuronal activity or the CSF acts as a signal transmitting medium to other areas within the CNS remains speculative.

Conclusions

Elevated EC levels in CSF of American Indians indicate an alteration of the central EC system in this obesity- and diabetes-prone population. We further report that the peripheral and central role of ECs and cognate AEs in human obesity may differ profoundly because peripheral (but not central) anandamide is most strongly associated with human adiposity. Finally, we show for the first time that central OEA is positively associated with metabolic rate.

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