Published in final edited form as:

Neurogastroenterol Motil. 2016 September ; 28(9): 1409–1418. doi:10.1111/nmo.12843.

Endocannabinoid-related lipids are increased during an episode of cyclic vomiting syndrome

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Abstract

Background—The endocannabinoid system (ECS) and the hypothalamic-pituitary-adrenal (HPA) axis are important neuromodulators of nausea and vomiting. This led us to hypothesize that patients with cyclic vomiting syndrome (CVS) have lower serum endocannabinoids (eCBs) and higher salivary cortisol and alpha amylase.

Methods—Serum eCBs and related lipids, *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamide (PEA), and salivary cortisol, and alpha amylase (index of sympathetic nervous system activity) were measured in 22 CVS patients (age 40±11, female=17) in the well and sick phases and 12 matched controls (age 37±12, female=10).

Results—Contrary to our hypothesis, serum concentrations of the eCBs were not different among the study groups. However, serum concentrations of OEA and PEA were significantly

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Author Contributions

TV participated in study concept and design, acquisition of data and analysis, interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, obtaining funding and study supervision

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Disclosures

All authors do not have any disclosures or conflicts of interest

higher during the sick than well phase in CVS patients ($p=0.001$ and $p=0.04$). There were positive correlations between serum PEA and nausea scores in the sick phase (Pearson's $\rho=0.48$, $p=0.036$) and between serum OEA and poor sleep quality in patients (Pearson's $\rho=0.7$, $p=0.0005$). Salivary cortisol and alpha amylase were not different between patients and controls, but subgroup analysis revealed that both were significantly higher in marijuana users compared to non-users during the sick phase ($p=0.04$ and 0.03 , respectively).

Conclusions—These data demonstrate that endocannabinoid-related lipids, OEA and PEA, are mobilized in the sick phase of CVS and are positively correlated with several of the symptoms of a CVS episode. These data also suggest the hypothesis that chronic marijuana use results in enhanced stress responses during CVS.

Keywords

palmitoylethanolamide; N-oleoylethanolamine; cannabinoids; marijuana; salivary cortisol; salivary alpha amylase

Introduction

Cyclic vomiting syndrome (CVS) is a functional disorder characterized by episodic vomiting. CVS consists of the asymptomatic well phase and the sick phase. The sick phase may start with a prodrome followed by the emetic phase. During the emetic phase, patients typically vomit multiple times over hours to days after which they recover.(1) The pathophysiology of CVS is unknown, although mitochondrial DNA polymorphisms and altered endocrine and/or autonomic stress responses have been implicated.(2-5) Recently, chronic marijuana use has been implicated in the etiology of a form of cyclic vomiting associated with compulsive hot water bathing, coined “cannabinoid hyperemesis syndrome.”(6) The treatment of CVS is mostly empiric and patients often resort to alternative treatments; about 40% of patients use marijuana for relief of symptoms.(7)

The active ingredient in marijuana - Δ^9 -tetrahydrocannabinol (THC) - produces its effects via binding to two G protein-coupled receptors named cannabinoid receptor subtypes 1 and 2 (CB1 and CB2).(8) Two endogenous CB1 receptor ligands, *N*-arachidonylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) (endocannabinoids, eCBs) have been identified; 2-AG also binds to the CB2 receptor.(9) AEA is one of a family of *N*-acylethanolamines (NAEs) that also includes *N*-oleoylethanolamine (OEA) and palmitoylethanolamide (PEA). (10, 11) Circulating concentrations of AEA, OEA and PEA are highly correlated (12), likely because they share common pathways of biosynthesis and degradation.(13) OEA and PEA do not bind to cannabinoid receptors; however, both are agonists of peroxisome-proliferator-activated receptor alpha (PPAR- α) and PEA also binds the G protein-coupled receptors, GPR55 and GPR119.(14)

Human experience with CB1 receptor agonists and antagonists support an important role for CB1 receptor tone in the regulation of nausea and vomiting.(15) The CB1 receptor agonist, nabilone, is efficacious in the treatment of chemotherapy-induced nausea and vomiting.(16) Conversely, nausea and vomiting are frequent adverse effects accompanying CB1 receptor antagonist use in humans.(17) An inverse relationship between circulating eCB

concentrations and the occurrence of motion sickness suggests that cannabinoid receptor activation exerts a tonic, inhibitory effect on nausea and vomiting.(18) Contrary to these findings, chronic marijuana use can worsen CVS symptoms(19) and can cause the purported “cannabinoid hyperemesis syndrome.(6)

These led us to hypothesize that an attenuated stress response with low endocannabinoid concentrations in the blood during the sick phase contributes to nausea and vomiting in CVS. The aim of our study was to examine circulating blood concentrations of eCBs and related lipid concentrations in CVS patients and controls to test two specific hypotheses: first, that eCB concentrations are lower in patients in the well phase compared to healthy controls; and second, that eCB concentrations are lower in the sick than the well phase. A secondary goal was to examine the correlation of circulating NAEs with salivary cortisol and salivary alpha amylase; measures of distress during a CVS episode; sleep quality and measures of anxiety and depression.

Methods

Study design

The study was approved by the Institutional Review Board at the Medical College of Wisconsin. Subjects gave their written informed consent prior to the study. CVS patients were recruited from a gastroenterology clinic at the Medical College of Wisconsin (MCW), which is a tertiary referral center for these patients. Age and gender matched controls were recruited with the use of fliers posted at MCW. One hundred and seventeen patients and fourteen controls consented to participate in the study. Paired blood and saliva samples (in both the sick and well phase) were obtained from 22 patients. A paired sample analysis was done to control for between subject variability that might otherwise obscure the within subject well vs. sick changes. Paired samples were not available in the remainder of patients either due to logistic reasons such as travel and unavailability of study staff over the weekends or because the patient did not have an episode of CVS during the study period (Figure 1). Of the 14 control subjects who consented, 2 dropped out and 12 completed the study. Patient demographics and clinical features were collected using a standard questionnaire. Blood and saliva samples were obtained from controls and from patients during a CVS episode/sick phase and the well phase. As there is a diurnal variation in both cortisol and endocannabinoids, an attempt was made to collect blood and saliva samples both in the morning and evening hours. To this end, samples were drawn between 0730 and 1100 hours and between 1330 and 1700 hours; however, there were no differences between morning and afternoon samples within each group. Because of this and missing samples due to clinical and logistic issues, we averaged the morning and afternoon results for each individual and condition if both were available and considered them for statistical analysis as one data point. During the sick phase, blood was collected in the emergency department, hospital or in the patient's home by an experienced phlebotomist. Samples were collected within 3.0 (range 0.1- 6.0) days of the onset of an episode.

During a CVS episode, patients were treated following standard medical practice and received IV fluids; antiemetics such as ondansetron, promethazine and diphenhydramine; benzodiazepines such as lorazepam; and narcotics such as hydromorphone for pain control

when needed.(5) These medications were administered intravenously in the hospital and were taken orally when patients were at home. Standard care was not withheld as we considered this to be unethical and prompt treatment with these medications can prevent progression of CVS symptoms and shorten or terminate an episode. A urine toxicology screen was performed in patients and controls to check for use of marijuana. Urine samples were not obtained in 2 patients.

Assessment of severity of CVS, sleep quality and presence of anxiety and depression

The Index of Nausea, Vomiting and Retching (INVR), a validated tool to measure the severity of symptoms, was completed by patients during a CVS episode. The INVR is a self-administered questionnaire consisting of an 8 item, 5 point Likert scale with tested reliability and validity. The INVR measures the patient's perceived (a) duration of nausea, (b) frequency of nausea, (c) distress from nausea, (d) frequency of vomiting, (e) amount of vomiting, (f) distress from vomiting, (g) frequency of dry heaves, and (h) distress from dry heaves. Subscale scores for total experience, occurrence and distress can be derived from the INVR. The range of possible scores for the subdomains of total experience is 0-32, total distress is 0-12 and occurrence is 0-20 with a higher score denoting increased severity of symptoms.(21-23) The severity of abdominal pain during an episode was measured using a visual analog scale on a scale of 1-10 with 10 being most severe.

Other parameters of interest such as the presence of anxiety and depression and quality of sleep were measured using the Hospital Anxiety and Depression Scale (HADS) and the Pittsburgh Sleep Quality Index (PSQI) respectively. The PSQI is a validated tool that measures seven domains of sleep consisting of sleep duration, sleep latency, daytime dysfunction, sleep efficiency, sleep disturbance and need to use medications to sleep.(24) There are 19 self-rated questions, which combine to form the seven domains of sleep. Each domain of sleep has a score of 0-3 and the sum of these scores yield a total global score which ranges from 0-21 with higher scores indicating worse quality of sleep.

Measurement of serum lipids

Blood samples were centrifuged within 60 min of collection, at $1000 \times g$ for 25 min, after which serum was collected and stored at -80°C until analysis. Deuterated 2-AG and AEA were added to the sera, followed by lipid extraction using solid phase columns as previously detailed.(25) Concentrations of AEA, 2-AG, PEA and OEA were quantified by isotope dilution following measurement using atmospheric pressure, chemical ionization liquid chromatography/mass spectrometry (LC-APCI-MS) as described previously.(25)

Measurement of salivary cortisol and alpha amylase

Saliva samples were collected from subjects using the Salivette (Plain Salivette, Sarstedt, Newton, NC). Study staff gave subjects detailed instructions about the appropriate collection of samples. Subjects were asked to avoid tooth brushing and flossing as well as eating for at least 15-30 minutes prior to specimen collection. They were also asked to remove any lipstick or balm that would interfere with appropriate sampling. Saliva samples were stored at -20°C . Salivary cortisol concentration was measured by ELISA.(26) Alpha amylase activity was measured with a kinetic enzymatic method (Salimetrics, State College, PA).

This method utilizes a chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose.(27) The enzymatic action of alpha amylase on this substrate yields 2-chloro-p-nitrophenol, which can be spectrophotometrically measured at timed intervals at 405 nm. The amount of alpha activity present in the sample is directly proportional to the increase in absorbance at 405 nm. Salivary cortisol and salivary alpha amylase were measured during the well phase and during episodes of CVS concomitantly with measurement of serum lipid concentrations.

Statistical analysis

Data were analyzed using Stata 10.0 (StataCorp, College Station, TX). All group values are expressed as mean $\pm$ SD. Univariate analysis of continuous and categorical variables was performed using the Student's t-test and chi-square tests, respectively. Comparison between sick and well phase values of salivary cortisol, alpha amylase and circulating lipids in patients was performed using a paired t-test. The decision to perform a paired sample analysis was made *a priori* to control for between subject variability that might otherwise obscure the change within each subject in the well vs. sick phases. Based on preliminary data, we expected a 30% decrease in endocannabinoid concentration between subjects and controls with a between-subject coefficient of variation of 40%. Using these criteria, 12 paired samples of CVS patients were needed to show a significant difference. The study was powered for this strict paired-sample requirement, as this was the primary focus of our study. Group comparisons of aforementioned variables were also performed between controls and patients in the well phase. Bivariate linear regression and Pearson's correlation tests were used to analyze cortisol, alpha amylase, and lipid concentrations; and PSQI and INVR scores. Multivariate logistic regression was done to identify predictors of disease exacerbation and treatment resistance. Variables associated with p-values < 0.2 on univariate analysis, were included in the multivariate logistic regression analysis in which p-values of less than 0.05 indicated statistical significance for independent predictors. Linear correlation scatter plots were drawn to examine the association between serum lipids and PSQI and INVR scores.

Results

Demographics and clinical characteristics of CVS patients and controls

There were a total of 22 patients with CVS who had both blood and salivary sampling in the sick and well phase and 12 age and gender matched controls. Table 1 shows their demographics and clinical characteristics. The majority of patients were female (17 [77%]), Caucasian (13 [59%]) and overweight or obese (15 [68%]), similar to the control population. Ten (45%) patients had a personal history of migraine headache and 8 (36%) had a family history of migraine headaches. Eight (36%) patients used marijuana for symptoms and had a positive urine toxicology screen. and 6 (22%) reported taking very hot showers or baths for symptom relief. Among those who used marijuana, 6 patients reported chronic marijuana use for many years preceding the onset of symptoms while 2 patients reported marijuana use after the onset of symptoms of nausea and vomiting. One of these patients was an occasional user and smoked about once in 3 weeks and the other used it at least thrice a week. Results of marijuana use were not available in 2 patients as they were unable to provide a urine

sample. None of the controls were current users of marijuana. The numbers (percentages) of patients using medications for control of CVS symptoms were: tricyclic antidepressants (TCA's) 13 (60%), topiramate 8 (36%), coenzyme Q10 3 (14%), and L-carnitine 4 (18%). The mean $\pm$ SD duration of disease was 9.7 ± 8.5 years. CVS patients were significantly more likely to have anxiety (63 % vs. 25%, $p = 0.002$) and depression (42% vs. 0%, $p = 0.02$) compared to controls. On multiple regression analysis, anxiety continued to be significantly different between patients and controls ($p = 0.02$).

Patients with CVS had significantly poorer quality of sleep as measured by the PSQI. They had increased sleep disturbance, increased latency to onset of sleep, daytime dysfunction, and needed medications to sleep more often when compared to controls (Table 2). The total PSQI score was significantly higher in patients (higher scores signify a poorer quality of sleep) compared to controls (10.7 vs. 5.3 , $p = 0.0008$). The severity of nausea, vomiting, and retching measured by the INVR was moderately severe during the sick phase and is depicted in Table 2. Visual analog scales for abdominal pain were obtained in 14 patients, with a mean $\pm$ SD score of 6.8 ± 2.2 during the sick phase.

Serum lipid concentrations in CVS patients and healthy controls

Concentrations of the endocannabinoids AEA and 2-AG, and the related lipids, OEA and PEA were not significantly different between healthy controls and patients during the well phase. Both OEA and PEA were significantly increased during the sick phase compared to the well phase (Figure 2). There was a trend towards an increase in serum AEA (1.7 ± 0.2 vs. 2.4 ± 0.4 , $p = 0.09$) and 2-AG (95.3 ± 29.0 vs. 164.1 ± 54.3 , $p = 0.09$) between the well and sick phases in the CVS patients; however, statistical significance was not reached.

Salivary cortisol and alpha amylase in CVS patients and healthy subjects

Salivary cortisol tended to be increased during the sick phase compared to the well phase in patients (16.2 ± 12.3 vs. 10.6 ± 7.0 nmol/L, $p = 0.06$). Salivary cortisol was slightly, but not significantly, higher in patients in the well phase compared to healthy controls (10.6 ± 7.0 vs. 6.7 ± 3.0 nmol/L, $p = 0.08$). There were no significant differences in salivary alpha amylase between the sick and well phase in patients ($p = 0.27$) or between patients in the well phase and healthy controls ($p = 0.11$).

Marijuana use affects lipids, salivary cortisol, and salivary alpha amylase

Subgroup analysis of patients with and without marijuana use revealed significant differences in activation of the HPA axis and the sympathetic nervous system between marijuana users and non-users in the sick phase. Both salivary cortisol and salivary alpha amylase were significantly higher in marijuana users compared to non-users in the sick phase (Table 3). Salivary cortisol and alpha amylase were not different between marijuana users and non-users during the well phase. Serum PEA concentrations were significantly higher in the well phase in marijuana users when compared to non-users (Table 3). There were no significant differences between serum concentrations of 2-AG, AEA, or OEA between users and non-users at baseline or in the sick phase.

Correlation between lipid concentrations and sleep quality, anxiety, depression and severity of CVS episodes

The values for correlation between the different domains of sleep and lipid concentrations are shown in Table 4. Post hoc analysis showed a strong, positive correlation between OEA concentration in the well phase in patients and sleep latency, need for medications to sleep and total sleep efficiency as measured by the total PSQI score. OEA concentration in the sick phase had a positive correlation with latency to sleep in patients (Figure 3). A positive correlation between OEA concentration and sleep quality (daytime dysfunction) was noted in controls though they had a significantly better quality of sleep when compared to patients. There was also a positive correlation between AEA in the well phase in patients and the total PSQI score as shown in Figure 3. There were no significant correlations between severity of vomiting or retching and any of the lipid concentrations (Table 5). There was a significant, positive correlation between the total occurrence score for nausea and PEA concentration in the sick phase as seen in Fig. 3, panel D (Pearson's $\rho=0.48$, $p=0.036$). There were no correlations between anxiety and depression scores and lipid concentrations in either patients or healthy controls. (Table 5)

Discussion

This study is the first to examine the relationships among circulating eCBs and related lipids and the symptoms of CVS. Since CB1 receptor activation may prevent nausea and vomiting (28) and eCBs are reduced in motion sickness, we hypothesized that low eCB tone contributes to the development of nausea and vomiting in CVS. This hypothesis led to two predictions: first, that circulating eCBs are lower in CVS patients in the well phase compared to controls, and second, that circulating eCBs are lower in the sick phase compared to the well phase of patients with CVS. Contrary to our hypotheses, there was no difference in the concentrations of the eCBs, AEA and 2-AG between controls and CVS patients in the well phase although the sample size of our study was small. Similarly, our hypothesis that concentrations of the eCBs would be lower during the emetic phase was also incorrect; in fact, concentrations of AEA and 2-AG tended to be increased in the sick compared to the well phase though this did not reach statistical significance. One reason for this could be a Type II error and the significant number of dropouts in our study. ECs may have been significantly higher in the sick phase with a larger sample size and needs to be explored in future studies. Increase in ECs in the sick phase is consistent with the existent literature that supports mobilization of endocannabinoids in response to stress.(12, 29) Interestingly, we found that serum concentrations of two other NAEs (OEA and PEA) were significantly increased in the sick phase compared to the well phase. PEA concentrations were positively correlated with the severity of nausea, suggesting a relationship between the distress of the emetic episode and mobilization of PEA.

Circulating OEA and PEA are elevated in the sick phase of CVS

Emesis is a physically and psychologically stressful process. (23) In our study, there was a trend to an increase in salivary cortisol concentrations during the sick phase. The findings from our study showing an increase in circulating concentrations of OEA and PEA during the sick phase in CVS patients are consistent with previous studies demonstrating that

members of the NAE family are increased in the circulation during physical and psychological stress.(30),(12) In the present study, PEA showed a strong, positive correlation with measures of distress, supporting the hypothesis that stress mobilizes the NAEs. Data from multiple studies suggest that the NAE responses to stress are independent of HPA axis and SNS activation.(31) The finding that OEA and PEA increase in parallel is likely due to the fact that shared mechanisms are involved in their synthesis and degradation. Our finding that OEA and PEA are elevated during the sick phase begs the question of their potential role in the symptoms of the disorder. Circulating concentrations of OEA are associated with reduced appetite,(32) and elevations in OEA during the sick phase is thus consistent with the decreased appetite that patients report clinically during an episode even after cessation of vomiting.

OEA has also been associated with sleep disorders; previous studies found elevated OEA concentrations in the circulation of individuals with sleep apnea(33) and in the CSF of individuals who are sleep deprived.(34) Preclinical studies support a role for elevated OEA concentrations in enhancing wakefulness, suggesting that OEA is increased during sleep disturbance and could function to maintain a state of wakefulness.(35) CVS patients exhibit significant sleep dysregulation, including long latencies to sleep and increased sleepiness during the day. Consistent with the existing literature, there was a significant, positive correlation between serum OEA concentrations in the sick phase and latency to sleep, leading to the hypothesis that elevated OEA concentrations contribute to the poor sleep quality during the emetic episode. Interestingly, sleep deprivation is often a trigger for emetic episodes (36) and inducing sleep with the use of sedatives has been shown to abort an episode of CVS.(5) PEA was also elevated in the sick phase in our study. PEA has also been shown to affect the sleep-wake cycle and increases wakefulness. (35) There are also data demonstrating that PEA reduces gastrointestinal motility.(37) Taken together, these findings suggest that stress elevates the family of NAE lipids and that they contribute to the loss of sleep, appetite and GI dysmotility that accompany a bout of emesis.

Increased OEA and PEA concentration in the sick phase of CVS may have both diagnostic and therapeutic implications. Cessation of vomiting is often used as a criterion for hospital discharge when in fact patients continue to be nauseated and are not ready to resume oral intake. OEA and PEA could potentially serve as surrogate objective markers for reduced appetite and nausea and could help monitor response to therapy in CVS patients even after vomiting has stopped. This may prevent early hospital discharges and subsequent readmissions and provide physicians with tools to guide management. Additionally, this also presents us with opportunities for novel pharmacological manipulation of these lipids to mitigate symptoms such as anorexia and sleep disturbance that are common during the emetic and recovery phases of CVS.

Effects of marijuana use

One third of the CVS patients in our study were marijuana users and data gained from comparing these individuals to CVS patients who do not consume marijuana has provided some interesting, preliminary insights. Although there were no differences in salivary cortisol or alpha amylase between users and non-users in the well phase, marijuana users

had significantly greater salivary cortisol and salivary alpha amylase concentrations during the sick phase than non-users. These results suggest that chronic exposure to cannabis results in increased reactivity of both the HPA axis and the SNS to stress. Given that CB1 receptor activation suppresses activation of the HPA axis to stress (38) and the SNS (39) and that chronic THC exposure can down regulate and desensitize CB1 receptors, (20) these data support the hypothesis that chronic marijuana use can result in exacerbated stress responses, and thereby could increase the frequency or intensity of emetic episodes in CVS. Indeed, chronic marijuana use has been implicated as a cause of cyclic vomiting.(40) Circulating concentrations of PEA were also higher in the well phase of CVS patients using marijuana compared to non-users. The significance of this difference is unclear. However, if the NAEs are a stress responsive system, these data provide further support for the hypothesis that marijuana use results in increased stress responsivity. Further studies are warranted to explore these findings and determine effects of chronic marijuana use on stress responses. This is particularly important given the widespread legalization and use of marijuana in the US.

This study has some limitations. The majority of patients (18 [81%]) had received some form of therapy including antiemetics, sedatives and, in some instances, narcotics before collection of samples. Salivary cortisol and salivary alpha amylase concentrations were significantly higher in marijuana users compared to non-users despite the use of opiates, which can attenuate the stress response. In fact, this only strengthens our findings as it is likely that these differences would be even greater without the use of opiates. Other limitations include the absence of a control group without symptoms of CVS but with use of marijuana in the past; this would have been helpful to determine if the stress response is altered mainly due to marijuana. We intend to include such groups in the future to address this important question of the effects of marijuana on stress responsiveness. The dropout rate in our patient population was high due to logistic difficulties associated with obtaining samples during an episode including travel and study staff availability. Additionally, some patients did not follow up during an episode, which is understandable given the severity of symptoms that patients can experience when they are sick. However, only paired samples were analyzed as this strategy would yield more accurate results by reducing within-subject variability that might otherwise confound the findings. Eight patients (36%) were on amitriptyline for prevention of CVS episodes and only 3 patients were not on any form of therapy at the time of sampling. The median delay from onset of symptoms to sample collection was 3 days (range 0.1- 6 days). Thus, our measurements may not necessarily be reflective of changes that occur immediately at the onset of a CVS episode. These limitations are unavoidable for logistical and ethical reasons. Despite this, our findings provide provocative insights into the response of the peripheral ECS during an episode of CVS.

In conclusion, we have demonstrated that circulating lipids, OEA and PEA are increased during the sick phase in patients with CVS. Based upon these and other findings, we hypothesize that the family of NAE lipids is increased in the circulation during stress and that they may contribute to several of the symptoms of the disease, particularly the anorexia and poor sleep quality. We did not find differences in the circulating concentrations of two lipids known to act as CB1 receptor agonists, which suggest that if low endogenous CB1

receptor tone contributes to CVS etiology, it is not mediated by low concentrations of the eCBs. In fact, our finding that marijuana users had a significantly increased activation of the HPA axis and the SNS during an episode compared to non-users suggests that CVS sufferers could have reduced CB1 receptor expression. Further studies examining CB1 receptor expression and signaling in CVS and a more comprehensive study of effects of marijuana use on the symptoms and clinical course of CVS are warranted.

Acknowledgements

The authors thank Dr. Daniel Bussye for use of the Pittsburgh Sleep Quality Index and Verna A. Rhodes for use of the Index of Nausea, Vomiting and Retching. We are also grateful to the staff at the Translational Research Unit at the Medical College of Wisconsin for their assistance.

Funding

This was supported by the Clinical and Translational Research Institute NIH grant no. UL1RR031973, the Research and Education Component of the Advancing a Healthier Wisconsin Endowment at the Medical College of Wisconsin and the Aurora Research Institute.

Abbreviations

CB1	Subtype 1 of the cannabinoid receptor
CB2	Subtype 2 of the cannabinoid receptor
CVS	cyclic vomiting syndrome
eCBs	endocannabinoids
ECS	endocannabinoid system
FAAH	fatty acid amide hydrolase
GPR55 and GPR119	G Protein-Coupled Receptors 55 and 119
HADS	Hospital Anxiety and Depression Scale
HPA axis	Hypothalamic-pituitary-adrenal axis
INVR	Index of Nausea, Vomiting and Retching
LC/MS	Liquid chromatography/mass spectrometry
MCW	Medical College of Wisconsin
NAEs	N-acylethanolamines
OEA	N-oleoylethanolamine
PEA	N-palmitoylethanolamide
PPAR-α	peroxisome-proliferator-activated receptor alpha
PSQI	Pittsburgh Sleep Quality Index
PVN	paraventricular nucleus

SON	supraoptic nucleus
TCA's	tricyclic antidepressants
THC	⁹ -tetrahydrocannabinol
TSST	Trier's Social Stress Test

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Key Points

- The endocannabinoid system and the hypothalamic-pituitary-adrenal axis play important roles in the neurobiology of nausea and vomiting.
- We examined the endocannabinoids and the HPA axis in patients with cyclic vomiting syndrome (CVS) during the sick and well phase and in comparison to normal subjects. Endocannabinoid-related lipids, N-oleoylethanolamine and N-palmitoylethanolamide were increased in the sick phase of CVS.
- Salivary cortisol and alpha amylase were also increased in the sick phase, but only in CVS patients who used marijuana, suggesting that marijuana may result in alteration of the stress response in CVS.

Figure 1.
Flow diagram of study procedures and recruitment in CVS patients

Figure 2. Serum concentrations of 2-AG (a), AEA (b), OEA (c), and PEA (d) (pmol/ml) in 12 controls and 22 CVS patients during the well and sick phase are shown. ** denotes a p value < 0.01 and * a p value of < 0.05

Figure 3.

Positive correlation between different domains of sleep quality and ECs in 20 CVS patients is shown (a) shows a significant correlation between the total PSQI score and serum concentration of OEA in the well phase, $\rho=0.7$, $p<0.0001$ (b) shows a significant correlation between sleep latency and serum concentration of OEA in the sick phase, $\rho=0.49$, $p=0.03$ (c) shows a significant correlation between the total PSQI score and serum concentration of AEA in the well phase in CVS patients, $\rho=0.45$, $p=0.04$ (d) shows a positive correlation between occurrence of nausea and ECs and serum concentration of PEA in the sick phase in 19 CVS patients. $\rho=0.48$, $p=0.036$

Table 1

Demographics and characteristics of CVS patients and controls

	Controls n=12	CVS patients n=22	p value
Age (mean±SD)	37±12	40±12	NS
Gender			
Female n (%)	10(83%)	17(77%)	NS
BMI (mean±SD)	28.6±1.3	29.6±1.5	NS
Race n (%)			0.32
White	10 (83%)	13(59%)	
Black	2 (17%)	8 (36%)	
Hispanic	0 (0%)	0 (0%)	
Other		1 (3%)	
Anxiety n (%)	3 (25%)	14 (63%)	
Anxiety score	4.9±3.0	10.0±4.0	0.002*
Depression n (%)	0 (0%)	8 (42%)	
Depression score (mean±SD)	1.6±1.4	6.8±5.0	0.002*
Marijuana use n (%)	0(0%)	8 (36%)	0.02*

Table 2

Sleep quality and severity of nausea, vomiting and retching in CVS patients and controls

	Controls n=12	CVS patients n=20	p value
Sleep scores (total PSQI score)^a	5.3±3.6	10.7±4.1	0.0008*
Sleep duration	0.4 ± 0.8	0.7 ± 1.1	0.45
Sleep disturbance	1.4 ± 0.7	2.0 ± 0.7	0.03*
Sleep latency	1.0 ± 0.9	2.1 ± 1.1	0.007*
Daytime dysfunction due to sleepiness	0.8 ± 0.8	1.6 ± 1.0	0.02*
Sleep efficiency	0.3 ± 0.7	1.1 ± 1.3	0.06
Overall sleep quality	1.0 ± 0.85	1.55 ± 0.88	0.09
Need medications to sleep	0.41 ± 0.99	1.75 ± 0.37	0.006*
INVR scores^b			
Total experience score		20.1±8.2	
Total occurrence score		11.7±4.4	
Total distress score		7.1±3.7	
Abdominal pain score^c	NA	6.8±2.2	

^a denotes total PSQI sleep scores which can range from 0-21 with 0 denoting excellent sleep and 21 the worst quality of sleep. The total PSQI is the sum of the seven domains of sleep.

^b denotes the severity of nausea, vomiting and retching determined by the Index of Nausea, Vomiting and Retching in patients

^c shows the severity of abdominal pain in CVS patients using the visual analog scale in the sick phase. (mean±SD)

Table 3

Endocannabinoid concentration, salivary Cortisol, and salivary alpha amylase in marijuana users and non-users with CVS

	Non users [^] n=12	Marijuana users [^] n=8	p value
2-arachidonoylglycerol (pmol/ml)			
Well phase	121.9±174.8	72.1±67.8	0.45
Sick phase	192.6±328.5	155.6±137.2	0.76
N-arachidonylethanolamine (pmol/ml)			
Well phase	1.6±0.9	2.0±1.3	0.38
Sick phase	2.9±2.9	2.0±0.7	0.41
N-oleoylethanolamine (pmol/ml)			
Well phase	2.1±2.0	3.0±1.5	0.29
Sick phase	3.5±2.9	3.7±2.2	0.85
N-palmitoylethanolamide (pmol/ml)			
Well phase	8.9 ± 5.0	17.5 ± 10.2	0.02*
Sick phase	14.5±8.6	22.8±23.3	0.27
Salivary cortisol (nmol/L)			
Well phase	7.9±4.4	12.7±9.2	0.13
Sick phase	9.9 ± 7.3	22.4±13.9	0.02*
Salivary alpha amylase (U/ml)			
Well phase	220.6±168.7	214.6±147.8	0.93
Sick phase	162.4 ± 131.2	356.0 ± 229.0	0.03*

[^] Data is presented on 20 CVS patients who were able to provide a urine sample to check for marijuana use. (mean±SD)

Table 4

Correlation between eCBs and domains of sleep in 20 CVS patients and 12 controls (Pearson correlation coefficient and p values are reported)

PSQI/eCBs	Sleep duration	Sleep disturbance	Sleep latency	Daytime dysfunction	Sleep efficiency	Sleep quality	Needs medications to sleep	PSQI Total
Sick 2-AG	0.56	0.10	0.95	0.10	0.06	0.48	0.37	0.53
Well 2-AG	0.49	0.34	0.34	0.48	rho=0.3 p=0.01	0.39	0.93	0.29
Control 2-AG	0.07	0.24	0.41	0.98	0.44	0.23	0.62	0.66
Sick AEA	0.84	0.97	0.15	0.57	0.44	0.97	0.19	0.18
Well AEA	0.57	0.71	rho=0.52 p = 0.02	0.93	0.07	0.05	0.25	rho=0.45 p=0.04
Control AEA	0.06	0.13	0.62	0.55	0.28	0.17	0.60	0.55
PSQI/eCBs	Sleep duration	Sleep disturbance	Sleep latency	Daytime dysfunction	Sleep efficiency	Sleep quality	Needs medication	PSQI Total
Sick OEA	0.18	0.92	rho=0.49 p=0.03	0.74	0.49	0.37	0.46	0.12
Well OEA	0.11	0.93	rho=0.72 p=0.0003	0.39	0.14	0.07	rho=0.48 p=0.03	rho=0.7 p=0.0005
Control OEA	p=0.89	p=0.50	p=0.32	rho=0.8 p=0.002	p=0.97	p=0.10	p=0.25	p=0.17
Sick PEA	p=0.11	p=0.09	p=0.63	p=0.51	p=0.63	p=0.60	p=0.16	p=0.88
Well PEA	p=0.22	p=0.16	p=0.60	p=0.77	p=0.58	p=0.68	p=0.96	p=0.46
Control PEA	p=0.86	p=0.94	p=0.33	p=0.32	p=0.71	p=0.28	r=0.5 p=0.02	p=0.24

Table 5

Correlation between eCBs and anxiety, depression and severity of CVS episodes as measured by INVR scores
(p values are reported)

	Anxiety	Depression	INVR
Sick 2-AG	0.67	0.67	0.99
Well 2AG	0.93	0.24	0.19
Control 2-AG	0.87	0.66	NA
Sick AEA	0.88	0.58	0.18
Well AEA	0.52	0.43	0.39
Control AEA	0.11	0.52	NA
Sick OEA	0.43	0.87	0.27
Well OEA	0.62	0.20	0.19
Control OEA	0.73	0.12	NA
Sick PEA	0.29	0.28	0.12
Well PEA	0.63	0.17	0.09
Control PEA	0.26	0.10	NA