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## Degradation of endocannabinoids in chronic migraine and medication overuse headache

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Chronic migraine (CM) is frequently associated with medication overuse headache (MOH). The endocannabinoid system plays a role in modulating pain including headache and is involved in the common neurobiological mechanism underlying drug addiction and reward system. Anandamide (AEA) and 2-arachidonoylglycerol are the most biologically active endocannabinoids, which bind to both central and peripheral cannabinoid receptors. The level of AEA in the extracellular space is controlled by cellular uptake via a specific AEA membrane transporter (AMT), followed by intracellular degradation by the enzyme AEA hydrolase (fatty acid amide hydrolase, FAAH). AMT and FAAH have also been characterized in human platelets.

We assayed the activity of AMT and of FAAH in platelets isolated from four groups of subjects: MOH, CM without MOH, episodic migraine and controls. AMT and FAAH were significantly reduced in CM and MOH, compared to either controls or episodic migraine group. This latter finding was observed in both males and females with CM and MOH. Changes observed in the biochemical mechanisms degrading endogenous cannabinoids may reflect an adaptive behaviour induced by chronic headache and/or drug overuse.

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**Keywords:** Endocannabinoids; Episodic migraine; Chronic migraine; Medication overuse headache

### Introduction

The natural history of migraine is still poorly understood, and some migraineurs remit with age whereas others progress to chronic migraine (CM) (Katsarava et al., 2004). The pathophysiology of headache chronification is very complex and involves impairment of trigeminal nociceptive processing, but also psychological and behavioural abnormalities. Epidemiological and clinical studies suggest a possible link between overuse of acute headache medication and CM (Olesen et al., 2006). Medication overuse headache (MOH) is increasingly recognized as a problem associated with considerable long-term morbidity and disability (Diener and Limmroth, 2004). MOH is at present the third most common phenotype of headache after tension-type headache and migraine. Yet, the pathophysiology of MOH remains unclear. An integrative hypothesis for compulsive reward seeking behaviour in MOH similar to that seen with substance dependence has been proposed (Calabresi and Cupini, 2005; Fuh et al., 2005). Interestingly, MOH has been recently found associated with reversible metabolic changes in some pain processing structures and with persistent orbitofrontal hypofunction even after withdrawal of analgesics (Fumal et al., 2006).

The endocannabinoid system plays a role in modulating pain (Pertwee, 2001; Wilson and Nicoll, 2002; Cravatt and Lichtman, 2003), and activation of type-1 cannabinoid (CB1) receptors has been shown able to inhibit trigeminal neurons whose activity has a critical role in migraine pathogenesis (Akermann et al., 2007). In addition, the endocannabinoid system is involved in the common neurobiological mechanism underlying drug addiction (Maldonado et al., 2006). Endocannabinoids modulate glutamatergic corticostriatal transmission and influence the activity of mesolimbic reward system (Maldonado et al., 2006). Anandamide (AEA) and 2-arachidonoylglycerol are the most biologically active endocannabinoids, which bind to both central and peripheral cannabinoid receptors. The level of AEA in the extracellular space is controlled by cellular uptake via a purported AEA membrane transporter

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Table 1  
Demographic and clinical characteristics of the population studied

Characteristics	MOH (n 30)	CM (n 21)	EM (n 28)	Controls (n 23)
Age, y	41±10	39±10	36±8	37±8
Gender, %				
Women	24 (80%)	14 (67%)	16 (57%)	16 (70%)
Men	6 (20%)	7 (33%)	12 (43%)	7 (30%)
Educational level				
Middle school or less	18 (60%)	16 (76%)	22 (79%)	22 (95.7%)
High school or degree	12 (40%)	5 (24%)	6 (21%)	1 (4.3%)
Nicotine dependence	11 (36.6%)	6 (28.6%)	10 (35.7%)	9 (39.1%)
Headache frequency, d/mo	22.1±5.6	19.5±4.2	6.3±2.6	–
Duration of headache, y	23±13.2	18±11	16±8.4	–

(AMT), followed by intracellular degradation by the enzyme fatty acid amide hydrolase (FAAH). AMT and FAAH have been also characterized in human platelets (Maccarrone et al., 2002; Cupini et al., 2006).

In the light of the hypothesized role of endocannabinoid system in headache control, as well as in some forms of substance abuse, the aim of the present study was to test the hypothesis that metabolism of endocannabinoids might be involved in CM and MOH. We therefore examined the activity of AMT and FAAH in platelets isolated from CM or MOH patients, compared with those of episodic migraine (EM) and control subjects.

## Subjects and methods

Study subjects consisted of a series of consecutive outpatients who complained of headache and were examined at the outpatient Neurological Clinic of S. Eugenio Hospital, Rome and Neurological Clinic of University of Perugia. The study protocol was approved by the local Ethics Committees and all patients gave their written consent to the study. Interviews were performed using a standard questionnaire. Information on age, gender, smoking habit, body mass index (BMI), educational level, employment status and headache type was recorded. Data on headache frequency, severity, duration as well as data on frequency and type of acute medications used were collected from headache diaries. Participants were required to fill a daily diary form providing information on headache attacks and medication intake for at least 1 month.

Twenty-one CM patients without MOH, 30 MOH patients (with a previous history of episodic migraine whose headache had markedly worsened during medication overuse), 28 EM and 23 healthy controls were recruited. Diagnosis of headache type was made according to the International Classification of Headache ICHD-II criteria and its revised version for CM and MOH, recently published on behalf of the International Headache Society (Olesen et al., 2006).

### Collection of blood samples

The study was conducted in a comfortable room, blood sample collection was performed by venopuncture from the antecubital vein between 08.30 and 09.30 h. Washed platelets were prepared from blood as reported (Maccarrone et al., 2002; Cupini et al., 2006), and were sent blind to the laboratory for biochemical analysis. All menstruated women included in the present study were tested during the same menstrual phase, i.e. the late follicular phase,

had regular menstrual periods, were not pregnant at enrollment, and were not under oral contraceptives treatment.

### Biochemical assays

The uptake of 400 nM 3 H-AEA (223 Ci/mmol, from NEN DuPont de Nemours, Köln, Germany) by AMT of intact platelets was measured as reported (Maccarrone et al., 2002; Cupini et al., 2006). The hydrolysis of 2.5 µM 3 H-AEA by FAAH (E.C. 3.5.1.4) activity was assayed in platelet extracts by reversed-phase high performance liquid chromatography (Maccarrone et al., 2002; Cupini et al., 2006).

### Statistical analysis

Descriptive statistics include: means (±2SD), variance, Standard error of the mean (SEM) and medians. Two-way ANOVA and Tukey test as post hoc analysis were used to compare the means of normally distributed continuous parameters (age, duration of chronic headache, number of days with headache/month, etc.) among patients and control groups. *P*-values less than 0.05 were considered significant.

Checking for the distribution fitting of AMT and FAAH values in control and patient groups, we verified that the samples in the comparison were not drawn from the same distribution (which was not normal) and had different medians. We therefore applied the Median test for the comparisons which frames the computation in

Table 2

Descriptive statistics of FAAH and AMT values and statistical significance among patient and control groups

	N°	Means (2SD)	SE	Variance	Median
<i>FAAH</i>					
Controls	23	33.65 (16.14)	3.36	260.69	44.00
EM	28	39.03 (23.07)	4.36	532.40	38.50
CM	21	12.37 (8.56)	1.86	73.33	10.00
MOH	30	10.67 (8.53)	1.55	72.79	7.00
<i>AMT</i>					
Controls	23	17.68 (8.16)	1.70	66.71	22.00
EM	28	20.92 (11.89)	2.25	141.54	21.15
CM	21	7.01 (4.86)	1.15	28.14	7.00
MOH	30	7.00 (5.30)	0.89	23.63	6.50

### Multiple Comparisons statistical significance

	Controls	EM	CM	MOH
<i>FAAH</i>				
Controls	–	0.6830	0.0001*	0.0002*
EM	–	–	0.0000*	0.0002*
CM	–	–	–	0.4004
MOH	–	–	–	–
<i>AMT</i>				
Controls	–	0.4376	0.0001*	0.0002*
EM	–	–	0.0003*	0.0002*
CM	–	–	–	0.6559
MOH	–	–	–	–

\*FDR controlling Multiple Comparison reject homogeneity at 0.0333 critical level.

Adjusted Bonferroni Multiple Comparison reject homogeneity at 0.00083 critical level.

terms of a contingency table. It simply counts the number of cases in each sample that fall above or below the common median, and compute the Chi-square value for the resulting  $2 \times k$  samples contingency table. We also used a multiple comparison approach based on the false discovery rate (FDR) controlling procedure for independent test statistics. FDR is the expected proportion of false rejections among all rejections and the power of this controlling procedure is uniformly higher than that of other multiple comparison methods (Benjamini and Hochberg, 1995). Error rate was also controlled using a Bonferroni correction.

## Results

Demographic and clinical characteristics of the study population are given in Table 1. The groups were similar with regard to age and gender. We did not observe significant differences among groups concerning smoking habit, education status and occupational condition. There was a significant difference concerning years of disease between EM and MOH ( $p < 0.05$ ), and number of days with headache per month between EM and either CM ( $p < 0.0001$ ), or MOH ( $p < 0.0001$ ). On the other hand, CM and MOH did not significantly differ with respect to the number of days with headache per month and years of disease. FAAH and AMT levels were significantly different among groups (Table 2). Median test revealed that FAAH and AMT activity values of CM and MOH patients are significantly lower than those of EM and control groups. No significant differences were found for AMT and FAAH levels between CM and MOH. No significant differences emerged in the comparison of FAAH and AMT activities between control and EM patient group ( $p = 0.6830$  for FAAH and  $p = 0.4376$  for AMT) (Table 2, Fig. 1). However, as we have previously observed (Cupini et al., 2006), FAAH and AMT activity levels were significantly higher in EM females with respect to females in the control group ( $p = 0.0339$  and  $p = 0.0012$ , respectively) and also with respect to females in CM ( $p = 0.0000$  for both FAAH and AMT) and MOH patient group ( $p < 0.0000$ , for both FAAH and AMT). Control females also showed significantly higher values of FAAH and AMT compared to females in CM patient group (FAAH:  $p = 0.0003$ , AMT:  $p = 0.0000$ ) and MOH patient group ( $p = 0.0000$  for both FAAH and AMT). Moreover, values of FAAH and AMT activities were in general lower in males than in females in all groups, with differences reaching the level of statistical significance for EM patient group

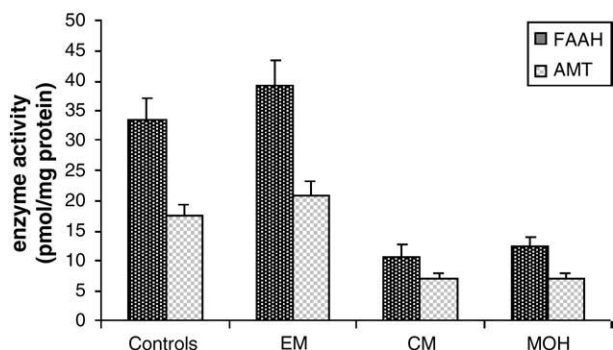


Fig. 1. AEA membrane transporter (AMT) and AEA hydrolase (fatty acid amide hydrolase, FAAH) activity in platelets of Controls, Episodic Migraine (EM), Chronic Migraine (CM), and Medication Overuse Headache (MOH). Both AMT activity and FAAH activity were expressed as pmol/min per mg protein, and the reported values represent the mean  $\pm$  S.E.

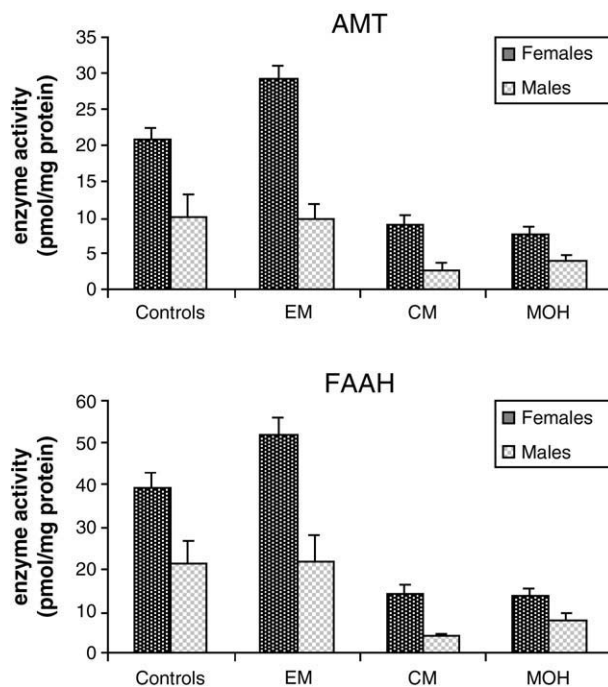


Fig. 2. AEA membrane transporter (AMT) (Upper part) and AEA hydrolase (fatty acid amide hydrolase, FAAH) (Lower part) levels in platelets of males and females in the four groups. Both AMT activity and FAAH activity were expressed as pmol/min per mg protein, and the reported values represent the mean  $\pm$  S.E.

(FAAH:  $p = 0.0023$  and AMT:  $p = 0.0001$ ) and CM patient group (FAAH:  $p = 0.0020$  AMT:  $p = 0.0050$ ) (Fig. 2). In control group significantly higher values of AMT were found ( $p = 0.0110$ ) whereas a trend toward slightly higher but not statistically significant value of FAAH in females than in males was found ( $p = 0.0618$ ). No significant differences were also found in MOH patients for both FAAH ( $p = 0.3613$ ) and AMT ( $p = 0.0679$ ). Finally, there was no significant difference among groups concerning the type of symptomatic drug used and FAAH and AMT activity levels.

## Discussion

The main finding of the present study is the observation that the degrading mechanisms of AEA are impaired in CM and MOH. We previously observed that female migraineurs have an increased activity of AEA hydrolase and AEA transporter suggesting an increased AEA degradation by platelets, which is consistent with a lowered endocannabinoid tone and possibly a reduced concentration of AEA in blood. This might reduce the pain threshold and possibly explain the prevalence of migraine in women (Cupini et al., 2006).

In the present study we have confirmed these findings demonstrating significantly higher median levels of both FAAH and AMT in females with EM compared with females in both control and CM groups.

We also found that FAAH and AMT activity in all groups were significantly lower in males than in females. However, our present findings suggest that the reduced FAAH and AMT activities observed both in CM and MOH do not seem to be related to

differences in gender, being confirmed in both sexes in these two chronic patient groups.

The observation that in CM and MOH there is lower platelet activity of FAAH and AMT, the two main mechanisms able to degrade anandamide, suggests an adaptative response induced by chronic headache and/or drug overuse. Interestingly, since there was no significant difference among groups concerning the type of symptomatic drug used and FAAH and AMT levels, we can argue that neither the overuse of symptomatic medications *per se* nor the intake of a specific class of symptomatic drug influence the FAAH and AMT levels. Conversely, it is possible that in both CM and MOH the chronic pain condition is associated with a down-regulation of the biochemical mechanisms degrading endogenous cannabinoids.

This mechanism, observed at the peripheral level, might be related to peripheral inflammation, but it cannot be ruled out that it also occurs within the central nervous system. In this context, it seems noteworthy that reduced levels of AEA in the CSF of CM and MOH have been recently found (Sarchielli et al., 2007). A possible link between this latter observation and the present study might be that a lowered anandamide level in CM induces a reduction in FAAH and AMT as an adaptative response. Alternatively, genetic factors may play a differential role in controlling AEA metabolism in the distinct populations studied. According to this genetic hypothesis, it has been shown that FAAH<sup>-/-</sup> mice possess 15-fold augmented endogenous brain levels of anandamide and display reduced pain sensation (Cravatt et al., 2001). Interestingly, a missense mutation in human FAAH is associated with drug abuse supporting a potential link among functional abnormalities in the endogenous cannabinoid system, drug abuse, and dependence (Sipe et al., 2002). On this basis, it is possible to speculate that different genetic backgrounds might play a role in the differential behaviour of the degrading machinery of anandamide in platelets of the studied groups.

An additional surprising finding of the present study is that decreased degrading mechanisms of endocannabinoids were observed not only in MOH patients but also in CM patients without MOH. Several explanations may account for this finding. However, a parsimonious interpretation is that the reduced AMT and FAAH activity in CM, although not yet associated with MOH, might render these subjects more vulnerable to medication overuse in their future natural history of headache.

We are aware that this study has some limitations such as the limited number of the included subjects and the fact that the enzyme activity was only analyzed in one tissue, platelets. Therefore, it seems to be too premature to speculate on specific disease mechanisms, which probably are more complex than those reflected by enzyme activity in platelets. We believe, however, that our analysis has also two major strengths, such as the support of a rigorous statistical controlling procedure and the

extremely careful selection of headache patients studied. Nevertheless, further studies are warranted to test our hypothesis.

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