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Short paper

Quantification of anandamide content in animal cells and tissues: the normalization makes the difference

Mauro Maccarrone*[1], Monica Bari[2] and Alessandro Finazzi-Agrò[2]

Address: [1]Department of Biomedical Sciences, University of Teramo, Piazza A. Moro 45, 64100 Teramo, Italy and [2]Department of Experimental Medicine and Biochemical Sciences, University of Rome Tor Vergata, Via Montpellier 1, I-00133 Rome, Italy

E-mail: Mauro Maccarrone* - Maccarrone@vet.unite.it; Monica Bari - Bari@Med.uniroma2.it; Alessandro Finazzi-Agrò - Finazzi@uniroma2.it

*Corresponding author

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Abstract

Anandamide (*N*-arachidonoylethanolamine, AEA) is an endogenous lipid that binds to cannabinoid receptors in the central nervous system and in peripheral cells. Quantitative analysis of AEA is generally based on the normalization to the fresh weight of the samples. Here, we show that the normalization procedure of AEA content is such a critical factor, that it might introduce *per se* significant discrepancies in the quantification of AEA even in the same sample. We suggest that a rapid, accurate and most reliable reference to quantify AEA and congeners from different sources is the protein content, a common parameter to cells and tissues.

Findings

Anandamide and the other "endocannabinoids" are endogenous ligands for "brain-type" (CB_1) and "spleen-type" (CB_2) cannabinoid receptors [1]. Also "non-classical" cannabinoid receptors and vanilloid receptors are activated by AEA [2], which plays a number of roles with potential clinical relevance, both in the central nervous system and in the periphery [1,3]. In view of the broad implications of AEA as central and peripheral modulator, its quantification is of utmost importance, also for the consideration that inhibition of AEA degradation via uptake and hydrolysis might open new therapeutic perspectives [1,3]. AEA content has been measured in pig, sheep and cow brain [4], in rat brain and rat peripheral tissues [5–7], in human brain and human cells in culture [8], and in mouse brain [9]. Most quantitative analyses of AEA content have been made by gas chromatography/mass spectrometry (GC/MS) after prepurification of the lipid extracts, and normalized to the fresh weight of the samples. This quantification has yielded concentration values in the range 0.2 – 30 pmol/g fresh weight [4–7]. Recently, we have developed a GC/MS procedure for the direct analysis of lipid extracts without prepurification steps [8,9]. Moreover, we normalized the amounts of AEA to the protein content of the samples, in order to better compare different tissues and cells, and also in consideration of the fact that determination of proteins is much more reliable than that of fresh weight. Using this direct GC/MS analysis we found concentrations of AEA in the 340 – 400 pmol/mg protein range [8,9]. Here, we show that, besides the procedure adopted, the normalization of AEA content is a critical factor, which might induce significant differences in the same sample. Most notably, we show how applying the same normalization procedure very similar or even identical amounts of AEA are found in tissues and in cells independently of their origin, suggesting that this compound is evenly distributed in animals.

AEA was determined in rat brain, mouse brain, human neuroblastoma CHP100 cells and human lymphoma

Table 1: Impact of Different Normalizations on the Quantification of Anandamide in Brain, and in Human Cells

Anandamide content	Rat brain	Mouse brain	Neuroblastoma CHP100 cells	Lymphoma U937 cells
pmol/mg protein	350 ± 70	400 ± 75	340 ± 65	400 ± 70
pmol/mg DNA	700 ± 98	800 ± 160	380 ± 70	440 ± 80
pmol/mg Pi[a]	1.4 ± 0.3	1.6 ± 0.3	2.1 ± 0.4	3.3 ± 0.7
pmol/mg FW[b]	14 ± 5.6	16 ± 6.4	27 ± 9.7	28 ± 10.5
pmol/10^6 cells	N.D.[c]	N.D.[c]	19 ± 4	18 ± 4

Values are reported as the mean ± S.D. of 6 to 8 independent determinations, each performed in duplicate. [a]Pi, lipid phosphorus; [b]FW, fresh weight; [c]N.D., not determined.

U937 cells [8,9], and its content was then normalized to 1 mg of protein, DNA or lipid phosphorus, to fresh weight, or to the cell number. Different aliquots of the same sample were used for these assays. While it is obvious that a different normalization method may yield very different values of AEA in the same sample (~12 to 50-fold lower when AEA was normalized to fresh weight or cell number, as compared to protein or DNA content), it is noteworthy that normalization to a common parameter yielded similar or even identical levels of AEA in all samples tested (Table 1). In particular, normalization to DNA content yielded the same amounts of AEA in rat and mouse brain, but approximately half that value in both tumor cells (Table 1). This finding seems in agreement with the fact that these cells are rapidly growing, and hence they have more DNA. Instead, normalization to fresh weight showed S.D. values of ~40%, in line with previous reports [4–7], whereas normalization to the other parameters showed S.D. values of ~15–20% only (Table 1). This observation might reflect the large variability of water content among the different samples.

Taken together, the present observation might represent a caveat to researchers when they report on the content of a metabolite, also by using newly developed liquid chromatography/mass spectrometry techniques [10]. On the other hand, the present results suggest that the most reliable reference to quantify AEA and congeners from different sources is the protein content. In fact, it is a parameter common to cells and tissues, which is easy and rapid to measure with accuracy and reproducibility, even in tiny samples.

Authors' contributions

MM conceived and coordinated the study. MB partecipated in sample preparation and assay. AFA partecipated in the study design and coordination.

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