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IDENTIFICATION AND QUANTIFICATION OF STEAROYLISOPROPYLAMIDE, A NEW ENDOGENOUS LIPIDIC MOLECULE IN BRAIN TISSUE

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Four different classes of endocannabinoids have been described: N-acyl-ethanolamides, acylamides, acyl-dopamines, acyl amino-acids. It is reasonable to assume that all these molecules represent a small part of a more complex lipidic-mediators-system which contributes to the regulation of cell functions and homeostasis.
While studying lipidic mediators, principally endocannabinoids, we have identified by GC/MS a new endogenous lipidic molecule: an unusual stearate derivative structurally related to endocannabinoids. This molecule, stearoylisopropylamine (SIPA) like palmitoylethanolamide (PEA), accumulated in brain tissue in which hypoxy condition were generated and in cultured cells where mitochondrial-uncouplig was induced. This may indicate the involvement of a specific metabolic pathway in the production of this molecule.

Amides of fatty acids, such as simple amides or ethanolamides derivatives of arachidonic, oleic, stearic, or palmitic acids constitute a large class of endogenous signaling lipids that, because of their structural similarity and modulation of several physiological processes, including inflammation and inflammatory-pain, mast cells degranulation, feeding behaviour, sleep etc., are classified as endocannabinoids[1].

Although several pharmacological effects of these lipids are reported to be mediated by the interaction of these molecules with the cannabinoid receptors CB1 or CB2, only few amides of fatty acids binds with appreciable affinity to cannabinoid receptors or interfere with degradation of endocannabinoids[2].

The existence of such large family of molecules endowed with different biological activities provides a hypothetical framework for a large and complex system of lipidic mediators that may be important for the function and regulation of endocannabinoid system or that operate in parallel *via* overlapping signaling pathways.

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derivative structurally related to endocannabinoids. This molecule, stearoylisopropylamine (SIPA) like palmitoylethanolamine (PEA), accumulated in brain tissue in which hypoxy condition were generated and in cultured cells where mitochondrial-uncouplig was induced indicating, although not jet identified, the involvement of metabolic pathways in the production of this molecule. Here we report a method for the simultaneous identification and characterisation, in mammalian tissue of PEA and SIPA.

RESULTS AND DISCUSSIONS

In an investigation devoted to cannabinoid identification and quantification in rat brain tissue, the presence of a new endogenous lipidic molecule was put in evidence. Its mass spectrum, reported in Figure 1, shows the molecular ion at m/z 325 and the primary fragments at m/z 296 and 282, corresponding to losses of $C_2H_5^{\bullet}$ and $C_3H_7^{\bullet}$ respectively. The most abundant ions are detected at m/z 86, 101 and 114 (**c**, **a**, **b** respectively), whose related structures are reported in the figure.

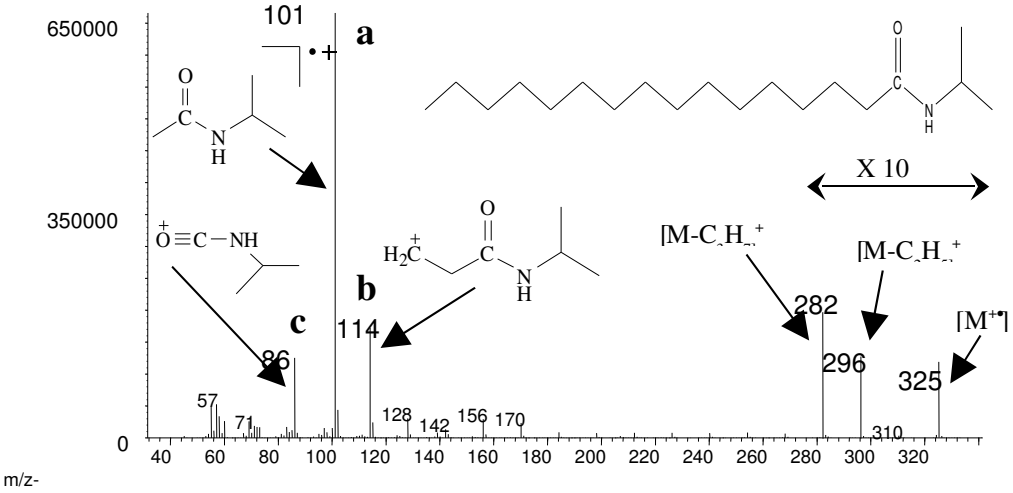

Figure 1. EI mass spectrum of stearoylisopropylamine

On the basis of these data, the structures of strearoylisopropylamine (SIPA) was preliminary assigned to this species and this hypothesis was confirmed by comparison with sintetized SIPA, showing the same retention time and the same mass spectrum.

The most of investigations carried out on naturally occurring cannabinoids were devoted to the quantification of PEA (Palmitoyl Ethanolamine). Some methods have been proposed for this aim, mainly based on GC/MS procedures[3-4].

On the contrary, any method was not reported in literature for the SIPA (Stearoylisopropylamine) quantification.

Looking at the biological relevance of this molecule, it was though of interest the development of GC/MS method for investigating its presence and its level in the rat brain tissue. Considering the high abundance of the ions at m/z 86, 101 and 114, they were employed for a possible single ion monitoring (SIM) method development. As discussed above, they are not related to the aliphatic chain, but can be justified by the structures **a**-**c** reported in Figure 1, all related to the imide containing moiety. Ion **a** is an odd electron ion, originating through a H rearrangement process, while the even electron ions **b** and **c** arise from simple bond cleavages.

First of all, the limit of detection was determined by injection of decreasing amount of standard SIPA. Considering that the expected values of SIPA concentration in the brain extract of interest would be in the range 10-50 pmol/μL, the lower concentration directly injected was 1 pmol/μL, which led to the SIM chromatograms reported in Figure 2a and 2b.

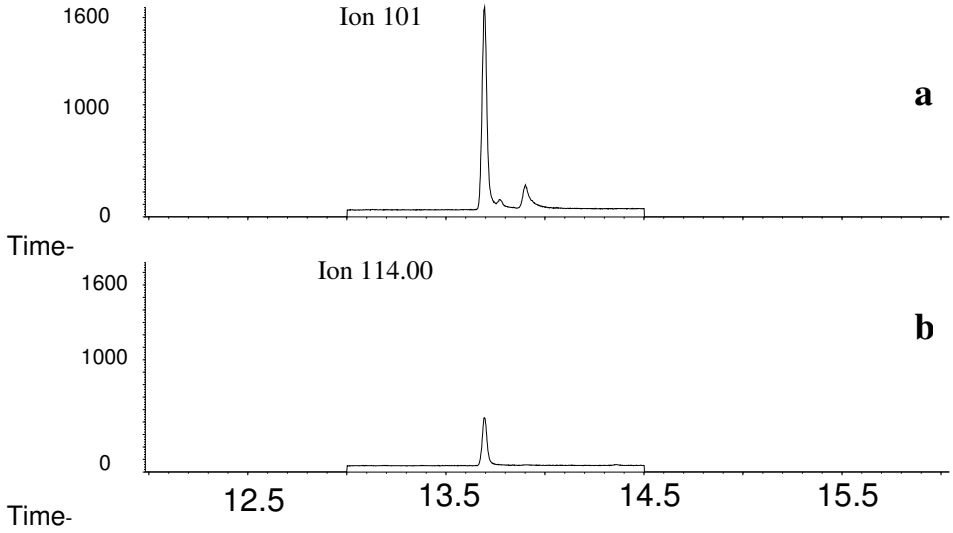

Figure 2. Single ion monitoring related to ions at m/z 101 and 114 obtained by injection of 1 μL of 1 pmol/μL solution of SIPA

A signal-to-noise ratio over 100 is present, showing that the method exhibit a sensitivity appropriate to the analytical problem. To obtain the calibration curve, Palmitoyl cyclopentylamine has been used as internal standard. This compound, strongly analogous to the analite of interest, leads to ions, at m/z 127 and 140, analogous to the **a** and **b** ones above described for SIPA. A further

ion is present at m/z 256 and originates from the primary loss of $C_5H_7^{\bullet}$, leading to an ammonium cation. This internal standard allowed to obtained the calibration regression lines for both SIPA and PEA. An equal quantity of i.s. was added to chloroform/methanol (2:1 *v:v*) solution at different SIPA and PEA concentrations (10-150 pmol/µL and 10-75 pmol/µL respectively).

The final internal standard concentration was in all cases 50 pmol/µL. The solution were injected, the relative peaks area were measured and the ratio [X]/[i.s.] was employed for the regression line calculation. The related equations were *Y=1.1706X–0.0656 (R^2=0.9985)* for SIPA and *Y=0.2135X-0.0114 (R^2=0.9913)* for PEA.

The method so developed was tested on a rat brain extract, leading to the data reported in figure 3. Levels of SIPA 8-10 fold higher than that of PEA have been determined.

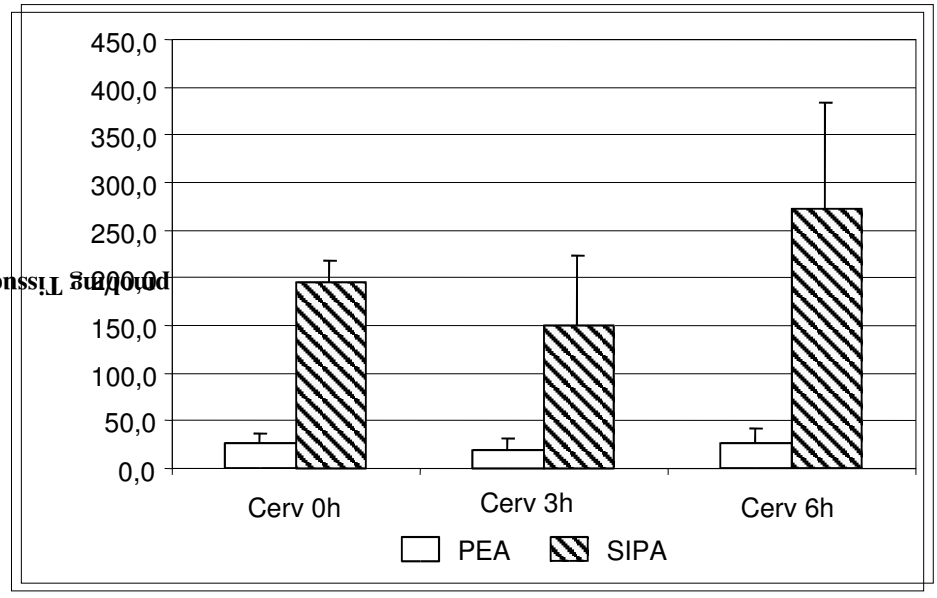

Figure 3. Amount of SIPA and PEA in biological lipids extracts in balb/c mouse brain at T=0, T=3 and T=6 hours post mortem

Research on other natural substrates are now in progress.

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