A hydrolysis-resistant fluorescent endocannabinoid analogue to investigate cellular endocannabinoid uptake and trafficking

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Endocannabinoids (ECs) are key mediators involved in many physiological and pathological conditions in CNS and peripheral tissues where they exert biological activities by interacting with extracellular and intracellular targets. By definition, ECs are endogenous molecules which activate primarily cannabinoid CB_1 and/or CB_2 receptors. Anandamide (AEA), N-arachidonoyl dopamine (NADA) and 2-arachidonoyl glyceryl ether (noladin ether; 2-AGE) are functionally more selective for CB_1; virodhamine appears to prefer CB_2 while 2-arachidonoyl glycerol (2-AG) is equipotent at both receptor subtypes (Bisogno, et al. 2005).

ECs effects are regulated by cellular biosynthesis, release, reuptake, trafficking and enzymatic metabolism. Unlike the clear knowledge about ECs biosynthetic and metabolic pathways, their cellular reuptake mechanism is not fully elucidated yet. The best experimentally supported theory relies on a passive membrane transporter-mediated mechanism. One of the main issues in elucidating the uptake process is the tight interplay between ECs plasma membrane movement and their rapid and almost complete cellular cleavage mainly dependent on FAAH and MAGL activity. Recently, we have shown that all ECs compete for the same putative membrane transporter (EMT) independently of their intracellular fate (trafficking and enzymatic inactivation) (Chicca et al. 2012). Thus, we have generated a fluorescent analogue of noladin ether (Fig. 1), the only hydrolysis-resistant EC.

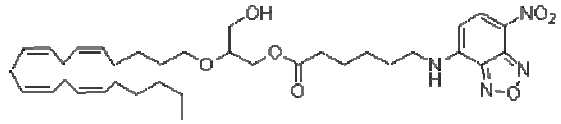

Fig. 1 NBD-AGE

We characterized NBD-2AGE properties by performing the classical radioactivity-based method and the analytical quantification (LC-MS). The results show that fluorescently-tagged noladin ether possesses the same biochemical features as noladin ether in terms of CB receptor binding, cellular uptake and trafficking, albumin binding and hydrolytic cleavage resistance. We made use of this new tool compound to study EC uptake and release kinetics in different cell types by FACS measurement. In addition, we also describe the movement of NBD-2-AGE from pre-loaded cells towards unloaded cells. Both processes could be selectively inhibited by the classic EMT inhibitors. When NBD-2AGE was co-incubated with AEA, 2-AG or noladin ether a selective competition in cellular uptake was detected. Other *N*-acetylethanolamines did not show any significant effect on the uptake as previously shown for the main endocannabinoids AEA and 2-AG (Maccarone et al. 2002, Jacobsson and Fowler, 2001 Chicca, et al. 2012). Altogether, our data suggest that NBD-2AGE is a very useful probe to investigate ECs cellular uptake and trafficking kinetics with a sensitive and radioactivity-free based method. Unlike the other ECs, NBD-2AGE is resistant to the fast and very efficient FAAH- and MAGL-mediated hydrolysis, which is a well-known confounding factor for studying cellular uptake and trafficking of AEA and 2-AG. Finally, NBD-2AGE will allow monitoring the ECs distribution in different cell types when applied to complex matrices.

References:

Bisogno T, et al. *Pharmacol Biochem Behav* 2005

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Maccarrone M, et al. *Biochem J* 2002