British Journal of Pharmacology (2008) 153, 179–181

0007–1188/08 $30.00
www.brjpharmacol.org

COMMENTARY

Good news for CB_1 receptors: endogenous agonists are in the right place

M Maccarrone[1,2]

[1]*Department of Biomedical Sciences, University of Teramo, Teramo, Italy and* [2]*European Center for Brain Research (CERC)/IRCCS S Lucia Foundation, Rome, Italy*

Endocannabinoids are endogenous ligands of brain-type (CB_1) and spleen-type (CB_2) cannabinoid receptors. *N*-Arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are prototype members of the fatty acid amides and the monoacylglycerols, two groups of endocannabinoids. Unlike CB_1, CB_2 receptors do not reside within 'caveolae', specialized membrane microdomains that are well-known modulators of the activity of a number of G protein-coupled receptors. In this issue of the *British Journal of Pharmacology*, Rimmerman and coworkers demonstrate that 2-AG is entirely localized in the caveolae of dorsal root ganglion cells, where also part of AEA ($\sim$30%) can be detected. However, most of AEA ($\sim$70%) was detected in non-caveolae fractions, that is where CB_2 receptors are localized. The different interaction of AEA and 2-AG with membrane microdomains might have significant implications for endocannabinoid-dependent autocrine and/or retrograde-paracrine signalling pathways. It also raises an important question about the structural determinants responsible for a different localization of two apparently similar endocannabinoids within lipid bilayers.
British Journal of Pharmacology (2008) **153,** 179–181; doi:10.1038/sj.bjp.0707566; published online 12 November 2007

Keywords: cannabinoids; caveolae; cholesterol; endocannabinoids; lipid rafts; signal transduction

Abbreviations: AEA, *N*-arachidonoylethanolamine (anandamide); 2-AG, 2-arachidonoylglycerol; CB_1, brain-type cannabinoid receptor; CB_2, spleen-type cannabinoid receptor; LRs, lipid rafts; QSAR, quantitative structure–activity relationship

Endocannabinoids are lipid signalling molecules that modulate several physiological processes. They are endogenous ligands of brain-type cannabinoid receptors (CB_1) and spleen-type cannabinoid receptors (CB_2), two G protein-coupled receptors that also bind Δ^9-tetrahydrocannabinol, the psychoactive component of *Cannabis sativa* (Howlett *et al*., 2002). *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are prototype members of two groups of endocannabinoids, the fatty acid amides and the monoacylglycerols respectively (Piomelli, 2003; Di Marzo and Petrosino, 2007).

The dependence of endocannabinoid signalling on 'lipid rafts' (LRs) is an emerging concept (Barnett-Norris *et al*., 2005; Dainese *et al*., 2007). LRs are specialized membrane microdomains that are enriched in cholesterol, sphingolipids and arachidonic acid and that have a tightly packed state (Hanzal-Bayer and Hancock, 2007). LRs are well-known modulators of the activity of a number of G protein-coupled receptors, for which a raft domain provides a more organized platform for the proper assembly of signalling complexes, also preventing cross-talks between different pathways. CB_1 receptors have been shown to reside within LRs (Bari *et al*., 2005), and consistently they co-localize with LR markers (Sarnataro *et al*., 2006). More recently, CB_1 receptors have been shown to co-localize with caveolin-1, a marker of 'caveolae' (Bari *et al*., 2007). These are considered a subclass of LRs represented by non-clathrin-coated and flask-shaped invaginations (diameter of $\sim$60–80 nm) in the plasma membrane. Unlike CB_1, CB_2 receptors do not reside within LRs or caveolae (Bari *et al*., 2006), as also shown by Rimmerman *et al*. (2008) in this issue of the *British Journal of Pharmacology*. These authors provide further information that seems to add a new player in the arena of the modulation of endocannabinoid signalling. In fact, they demonstrate by liquid chromatography/tandem mass spectrometry that 2-AG is concentrated in the caveolae of dorsal root ganglion cells where it co-localizes with components of the diacylglycerol pathway responsible for 2-AG production. Instead, AEA was detected in LRs and non-LR fractions to comparable levels (Rimmerman *et al*., 2008). Therefore, much similar cannabinoid receptors (CB_1 versus CB_2), their endogenous ligands (AEA versus 2-AG) show a different interaction with LRs, an observation that might have significant implications for endocannabinoid-dependent

Correspondence: Professor M Maccarrone, Department of Biomedical Sciences, University of Teramo, Piazza Aldo Moro 45, Teramo 64100, Italy.
E-mail: mmaccarrone@unite.it
Received 27 September 2007; accepted 10 October 2007; published online 12 November 2007

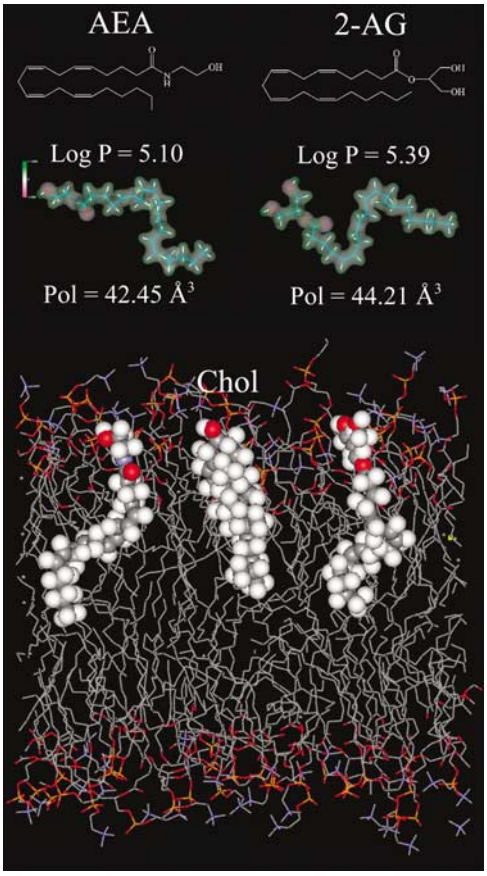

Figure 1 AEA versus 2-AG. The chemical structures, lipophilicity (log *P*), electrostatic potential (violet −1; green +1) and polarizability (Pol) values of AEA and 2-AG are shown in the upper panel. Both log *P* and Pol values were calculated by means of the HyperChemTM 6.03 molecular modelling system (Hypercube, Inc., Gainesville, FL, USA), as reported (Dainese *et al*., 2005). A schematic representation of (from left to right) AEA, cholesterol (Chol) and 2-AG embedded within a dipalmitoylphosphatidilcholine bilayer is shown in the lower panel. Here, atoms in the space-filling models were coloured with the following codes: oxygen in red, nitrogen in violet, carbon in grey and hydrogen in light grey. The figure was kindly provided by Dr. Enrico Dainese (University of Teramo, Teramo, Italy).

autocrine and/or retrograde-paracrine signalling. On one hand, the findings presented by Rimmerman *et al*. (2008) demonstrate that the CB_1 receptor agonists 2-AG and, to a lesser extent, AEA are present where they should be in order to activate their target: next to it, so that they can easily reach the binding site by lateral diffusion. On the other, these new data call for a reconsideration of the general concept that 2-AG is the only true agonist of CB_2 receptors whereas AEA (a weak and partial ligand for this receptor) does not have any physiological relevance (Sugiura *et al*., 2000). In fact, AEA might be localized more likely than 2-AG next to CB_2 receptors in dorsal root ganglion cells and possibly in other cell types.

The paper by Rimmerman *et al*. (2008) raises another important question: 'what are the structural determinants responsible for the different localization of AEA and 2-AG within lipid bilayers?' Like many other bioactive lipophilic molecules, endocannabinoids partition into membranes where they assume a thermodynamically favourable orientation and location. Quantitative structure–activity relationship (QSAR) studies have demonstrated that AEA and 2-AG share similar values of molecular descriptors like lipophilicity (expressed as logarithm of the octanol–water partition coefficient, log *P*), distribution of electrostatic potential and polarizability (Dainese *et al*., 2005). QSAR data were in accordance with the high flexibility of AEA observed by molecular dynamics simulations, which also showed that AEA embedded within the lipid bilayer tends to adopt a more extended conformation (Barnett-Norris *et al*., 2002). On the basis of the similarities of the molecular descriptors derived from QSAR analysis, it seems that 2-AG is embedded in the lipid bilayer in the same manner as AEA (Figure 1). Therefore, it is rather unexpected that these two endocannabinoids partition quite differently in raft and non-raft fractions. Maybe cholesterol, that is known to bind with AEA (Biswas *et al*., 2003), binds with 2-AG even better, presumably due to a better interaction with its acyl chain (Figure 1). As a consequence, cholesterol may favour the concentration of 2-AG within LRs. However, the study by Rimmerman *et al*. (2008) underlines the need for extensive investigations into the structural determinants that drive the interaction of apparently similar endocannabinoids with the surrounding lipid environment. Furthermore, the dependence of CB_1 receptors on LRs integrity makes it challenging from the therapeutic point of view to selectively target CB_1-dependent pathologies by means of LRs-oriented drugs. On a final note, the different interaction of AEA and 2-AG with raft and non-raft microdomains, along with the different localization of CB_1 and CB_2 receptors within these fractions, might represent a novel paradigm of ligand–receptor interactions whereby a third player comes into the game—the membrane lipids.

References

Bari M, Battista N, Fezza F, Finazzi-Agrò A, Maccarrone M (2005). Lipid rafts control signaling of type-1 cannabinoid receptors in neuronal cells. Implications for anandamide-induced apoptosis. *J Biol Chem* **280**: 12212–12220.

Bari M, Oddi S, De Simone C, Spagnuolo P, Gasperi V, Battista N *et al.* (2007). Type-1 cannabinoid receptors colocalize with caveolin-1 in neuronal cells. *Neuropharmacology*, in press.

Bari M, Spagnuolo P, Fezza F, Oddi S, Pasquariello N, Finazzi-Agrò A *et al*. (2006). Effect of lipid rafts on CB2 receptor signaling and 2-arachidonoyl-glycerol metabolism in human immune cells. *J Immunol* **177**: 4971–4980.

Barnett-Norris J, Hurst DP, Lynch DL, Guarnieri F, Makriyannis A, Reggio PH (2002). Conformational memories and the endocannabinoid binding site at the cannabinoid CB1 receptor. *J Med Chem* **45**: 3649–3659.

Barnett-Norris J, Lynch D, Reggio PH (2005). Lipids, lipid rafts and caveolae: their importance for GPCR signaling and their centrality to the endocannabinoid system. *Life Sci* **77**: 1625–1639.

Biswas KK, Sarker KP, Abeyama K, Kawahara K, Iino S, Otsubo Y *et al.* (2003). Membrane cholesterol but not putative receptors mediates anandamide-induced hepatocyte apoptosis. *Hepatology* **38**: 1167–1177.

Dainese E, Gasperi V, Maccarrone M (2005). Partial QSAR analysis of some selected natural inhibitors of FAAH suggests a working hypothesis for the development of endocannabinoid-based drugs. *Curr Drug Targets CNS Neurol Disord* **4**: 709–714.

Dainese E, Oddi S, Bari M, Maccarrone M (2007). Modulation of the endocannabinoid system by lipid rafts. *Curr Med Chem* **14**: 2202–2215.

Di Marzo V, Petrosino S (2007). Endocannabinoids and the regulation of their levels in health and disease. *Curr Opin Lipidol* **18**: 129–140.

Hanzal-Bayer MF, Hancock JF (2007). Lipid rafts and membrane traffic. *FEBS Lett* **581**: 2098–2104.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, *et al*. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* **54**: 161–202.

Piomelli D (2003). The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* **4**: 873–884.

Rimmerman N, Hughes HV, Bradshaw HB, Pazos MX, Mackie K, Prieto AL *et al*. (2008). Compartmentalization of endocannabinoids into lipid rafts in a dorsal root ganglion cell line. *Br J Pharmacol* **153**: 380–389 (this issue).

Sarnataro D, Pisanti S, Santoro A, Gazzerro P, Malfitano AM, Laezza C *et al*. (2006). The cannabinoid CB1 receptor antagonist rimonabant (SR141716) inhibits human breast cancer cell proliferation through a lipid raft-mediated mechanism. *Mol Pharmacol* **70**: 1298–1306.

Sugiura T, Kondo S, Kishimoto S, Miyashita T, Nakane S, Kodaka T *et al*. (2000). Evidence that 2-arachidonoylglycerol but not *N*-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. *J Biol Chem* **275**: 605–612.