Discrimination between Two Endocannabinoids

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Monoacylglycerol lipase (MAGL) deactivates 2-arachidonoylglycerol (2-AG), an endogenous ligand of cannabinoid receptors (endocannabinoid). In this issue of *Chemistry & Biology*, Chang and colleagues report an extremely specific MAGL inhibitor that should be useful to distinguish between actions of 2-AG and anandamide, another endocannabinoid.

2-Arachidonoylglycerol (2-AG) and arachidonoylethanolamide (anandamide) are two representative endogenous ligands of cannabinoid (CB) receptors and are collectively referred to as endocannabinoids (Pertwee et al., 2010). Structurally, both compounds are arachidonic acid-containing lipid molecules, and this similarity explains their common function as CB receptor agonists. However, there are several essential differences in terms of their in vivo roles. First, 2-AG is a full agonist of the central receptor CB1 as well as the peripheral receptor CB2, whereas anandamide is a partial agonist of both receptors (Sugiura et al., 2006). Next, in animal tissues, endogenous 2-AG level is generally a few orders of magnitude higher than anandamide level. In accordance with this fact, 2-AG is a major product of the phospholipase Cβ - diacylglycerol lipase pathway, which is most likely initiated by 1-stearoyl-2-arachidonoyl-phosphatidylinositol (Figure 1). On the other hand, anandamide is a minor component of fatty acyl ethanolamide (*N*-acylethanolamine) species in most animal tissues. Here, major species, such as palmitoylethanolamide, oleoylethanolamide, and stearoylethanolamide, don't affect CB receptors. All proposed anandamide biosynthesis metabolic pathways appear to generate not only anandamide but also other *N*-acylethanolamine species, and anandamide is a minor component because the fatty acyl moiety originates exclusively from the *sn*-1 position of glycerophospholipids where the arachidonic acid chain is present in a few percent or less (Ueda et al., 2010). Palmitoylethanolamide and oleoylethanolamide are well known to be agonists of peroxisomal proliferator-activated receptor α and several other receptors and exert various biological actions such as analgesic, anti-inflammatory, neuroprotective, and appetite-suppressing effects. Thus, even though anandamide exhibits distinguishing cannabimimetic activities in vivo, anandamide might be a byproduct concomitant with the generation of major *N*-acylethanolamines. Furthermore, recent studies demonstrate that 2-AG is released from postsynaptic neurons in response to depolarization and stimulation of Gq protein-coupled receptors and mediates retrograde synaptic suppression through the CB1 receptor found at presynaptic terminals (Kano et al., 2009). However, physiological significance of anandamide in the central nervous system is not fully elucidated. The final point of difference between 2-AG and anandamide is reflected by the difference in their CB receptors' independent functions; 2-AG potentiates $GABA_A$ receptor, whereas anandamide is one of the vanilloid receptor, TRPV1, agonists (Piscitelli and Di Marzo, 2012).

2-AG is principally deactivated by hydrolysis to arachidonic acid and glycerol by monoacylglycerol lipase (MAGL), whereas anandamide is hydrolyzed to arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH) (Figure 1). The latter enzyme also hydrolyzes all *N*-acylethanolamines, including palmitoylethanolamide and oleoylethanolamide. Over the last several years, specific inhibitors of these two enzymes have attracted much attention not only as pharmacological tools to elevate endogenous levels of endocannabinoids, but also as potential therapeutic agents for various diseases such as pain, inflammation, anxiety, and cancer (Feledziak et al., 2012). Since the inhibitors selective for MAGL over FAAH could be useful to discriminate biological actions of 2-AG from those of anandamide and other *N*-acylethanolamines, complete selectivity is essential to detangle these effects. However, achieving the selectivity is not so easy because of the similarity between the catalytic mechanisms of both enzymes, based on a serine nucleophile and similarity between 2-AG and anandamide chemical structures. One of the recently developed compounds, JZL184, modifies the serine nucleophile of MAGL by covalent carbamylation and is one of the most promising lead MAGL inhibitors (Long et al., 2009). However, even JZL184 partially inhibits FAAH when the high dose is repeatedly used. Moreover, ABHD6 and ABHD12, two other serine hydrolases, are also partially responsible for 2-AG degradation in the brain (Blankman et al., 2007). Hence, one must consider the cross-reactivity of MAGL inhibitors not only with FAAH, but also with ABHD6, ABHD12, and many other serine hydrolases, including carboxylesterases.

In this issue of *Chemistry & Biology*, Chang et al. (2012) report development of a class of *O*-hexafluoroisopropyl carbamates as improved MAGL inhibitors. Among them, KML29, which is structurally similar to JZL184, is demonstrated to be an extremely selective MAGL inhibitor both in vitro and in vivo. KML29 exhibited potent inhibition of mouse, rat, and human MAGL with IC_{50} values in 5.9–43 nM range but no detectable inhibition of FAAH from the same animal species (IC_{50}, >50 μM). The compound was also less inhibitory for ABHD6 (IC_{50}, 1.6–4.87 μM). Notably, the structure of hexafluoroisopropanol as a leaving group resembles the glycerol moiety of the substrate 2-AG. This may at least partly explain the high selectivity for MAGL. Treatment of mice and rats

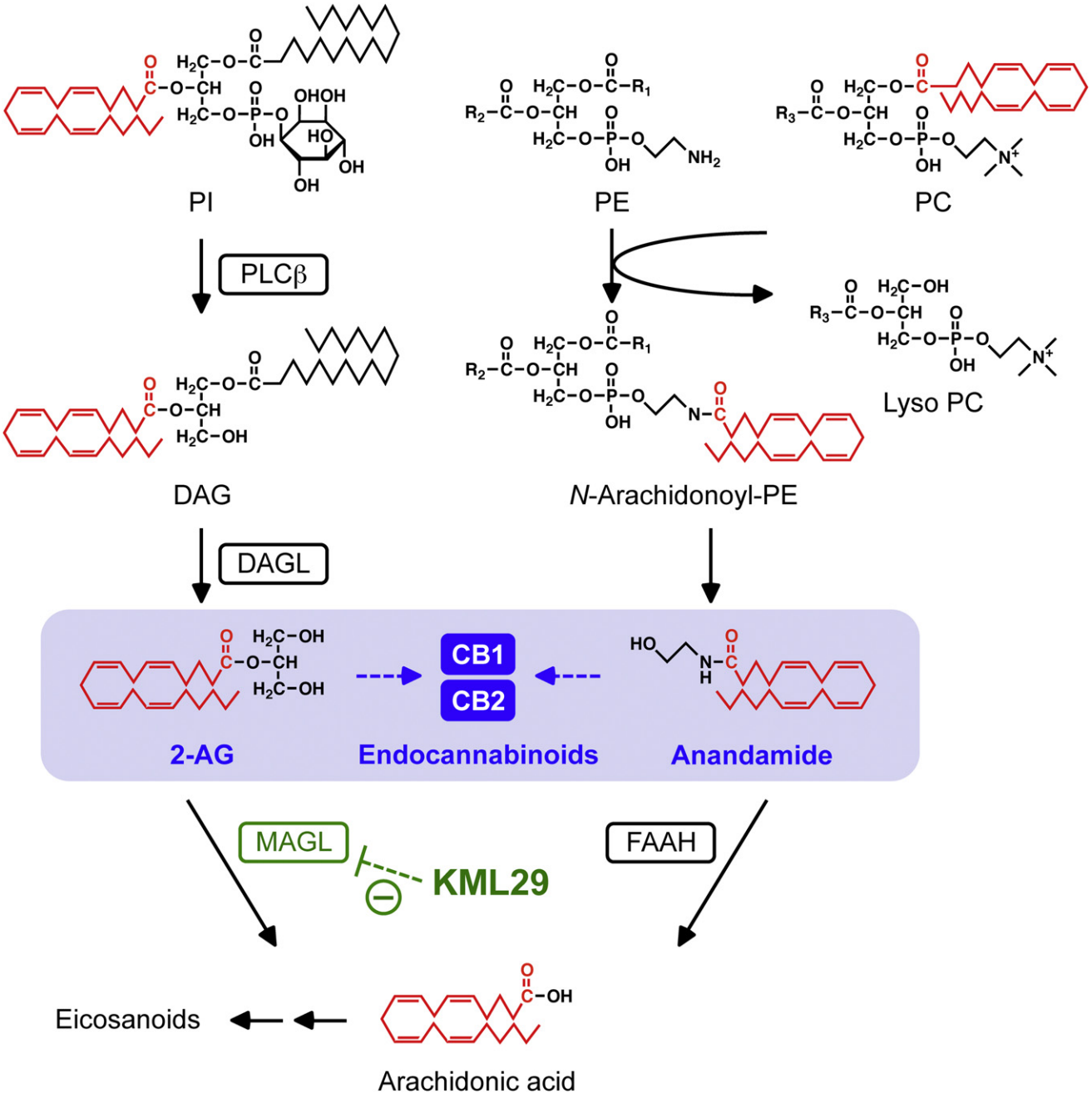

Figure 1. Major Metabolic Pathways of Two Endocannabinoids
DAGL, diacylglycerol lipase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PLCβ, phospholipase Cβ.

with KML29 dose-dependently elevated brain 2-AG level up to ~10-fold without alteration in brain levels of anandamide, palmitoylethanolamide, and oleoylethanolamide. KML29 also significantly lowered endogenous level of free arachidonic acid, an MAGL product, which may affect eicosanoid biosynthesis. These results strongly suggest that KML29 and related compounds represent excellent chemical probes to explore the physiological roles of 2-AG as well as MAGL.

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Breaking Down Order to Keep Cells Tidy

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Small heat shock proteins form large assemblies that protect cytoplasmic components when stressed. In this issue of *Chemistry & Biology*, Stengel et al. show that disturbing oligomer symmetry allows weak interfaces to catch intact substrate dimers.

Heat shock proteins provide quality control by recognizing and dealing with errors in the expressed proteome. This vital function contributes to cellular robustness conferring tolerance to the stresses of life by preventing cells, organelles, and tissues from becoming clogged with aggregated protein junk (Tyedmers et al., 2010). Heat shock proteins usually perform this function by acting as molecular chaperones, binding to nonnative polypeptide chains and unfolding/refolding the chain using energy stored in ATP. Members of the "small heat shock proteins" family have a short polypeptide sequence (molecular mass ~20 kDa), but they assemble into very large but polydisperse oligomers that undergo rapid subunit exchange and equilibrium dissociation. Although polydispersity has hindered the structural biology of small heat shock proteins, it is likely key to their function. Stengel et al. (2010) have previously employed a two-stage nanoelectrospray mass spectrometry technique (nanoES MS) whereby a spectrum of distributions of macromolecular assemblies within a polydisperse ensemble is measured first, followed by precise but arduous stoichiometric measurements of components of individual assemblies. In the new work published here they replace the second experimental step with modeling from the one dimensional data using newly developed algorithms, rendering the methodology suitable for high throughput applications.

NanoES MS can measure the mass of single assemblies in the gas phase under conditions that simulate stress, such as heat. Using this new technique, the small heat shock protein under study by Stengel et al. (2012) in this issue of *Chemistry & Biology* is from peas. Plants must withstand a wide range of temperatures, and it has been shown that at laboratory ambient temperature, the pea small heat shock protein is a dodecamer. The crystal structure of a closely related dodecameric small heat shock protein from wheat showed it was symmetrically built from six dimers (van Montfort et al., 2001). Previously, nanoES MS techniques revealed that the pea dodecamer rearranged to a polydisperse distribution of higher-order oligomers, with a preference for an even number of monomers, coincident with it becoming an active chaperone binding thermally unstable protein substrate (Stengel et al., 2010). In agreement with the two-dimensional experimental approach, the new method described here (Stengel et al., 2012) showed that for a given ratio of chaperone to a monomeric substrate (luciferase), the most highly populated complex assembly comprised 18 chains of small heat shock protein to 1 chain of luciferase.

Symmetric protein assemblies require strict geometric constraints between complementary, often interwoven, interfaces, and they can be visualized using PDBePISA (http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html) in all their variety (Krissinel and Henrick, 2007). Changing from a regular oligomer into a polydisperse ensemble is expected to lead to an increase in exposure of interface regions, and these rearrangements likely result in the presentation of protein surfaces ready to engage with destabilized substrates. However, most small heat shock proteins across all kingdoms of life are innately polydisperse. In fact, for the first described member, α-crystallin from the eye lens, assembly polydispersity confers solubility and transparency (Clark et al., 2012) and requires some level of activation to increase its affinity for denatured substrates. It is likely, then, that regulated exposure of chaperone binding regions for misfolded protein substrates is linked to conformational change in both substrate and chaperone. In the ATP-driven machines such as HSP60 chaperone, conformational change is driven by ATP-binding and hydrolysis, leading to substrate binding and release (Clare et al., 2012). The ability of small heat shock proteins to manage without ATP binding is in keeping with the energetically cheap exposure of binding sites in these flexible oligomeric assemblies. The new nanoES MS work now embellishes this view with the finding