Decoding endocannabinoid signaling

Lawrence J Marnett

Development of an inhibitor of an endocannabinoid-degrading enzyme provides insights into the role of 2-arachidonoylglycerol in the nervous system.

Endocannabinoids—the endogenous ligands for the cannabinoid receptors—are attracting increasing attention as mediators of neurological, anti-inflammatory and proliferative effects[1]. There are two well-characterized endocannabinoids: arachidonoylethanolamide (AEA or anandamide) and 2-arachidonoylglycerol (2-AG). 2-AG binds to the two cannabinoid receptors, CB1 and CB2, whereas AEA binds only to the CB1 receptor. CB1 and CB2 are seven-transmembrane G protein–coupled receptors that lower cyclic AMP levels following ligand binding. The CB1 receptor is abundant in the brain and the CB2 receptor is abundant in macrophages, but both receptors are being found in an increasing number of tissues. The brain CB1 receptor functions in a number of neurological responses, including postsynaptic desensitization of neuronal firing, which is mediated by 2-AG (**Fig. 1**).

Both AEA and 2-AG are generated on-demand and then rapidly inactivated by hydrolysis. The ephemeral nature of these mediators makes studies of their role in complex biological responses challenging. Genetic deletion or pharmacological inhibition of the hydrolases that inactivate AEA and 2-AG are powerful approaches to defining endocannabinoid biology. Knockout animals and specific inhibitors of fatty acid amide hydrolase (FAAH) have been instrumental in elucidating the role of AEA in cell signaling[2,3]. Comparable reagents have not been available for monoacylglycerol lipase (MAGL), which inactivates 2-AG, but in this issue, Long *et al.* report the first potent and specific inhibitor of this enzyme[4].

The most daunting aspect of generating useful reagents is maintaining specificity as one increases potency. In many cases, the specificity of an inhibitor is inversely proportional to the number of enzymes against which it has been screened. Long *et al.* tackled the problem of specificity up-front by screening candidate compounds from a

library of carbamates against a mouse brain membrane proteome that contains a range of serine hydrolases rather than against pure MAGL. They used activity-based protein profiling (ABPP) to covalently modify all the serine hydrolases with a fluorescent or biotinylated fluorophosphonate and screened for compounds that could selectively prevent labeling of MAGL. ABPP was used at each step of optimization to ensure that as more potent inhibitors were synthesized, they retained high

selectivity. The best compound in the series, JZL184, is a covalent inactivator that exhibits a half-maximal inhibitory concentration (IC_{50}) for MAGL of 8 nM (its IC_{50} for FAAH is 4 μM). JZL184 doesn't inhibit any of 40 other serine hydrolases in the mouse brain membrane proteome. When administered to mice, JZL184 inhibits MAGL activity by 85% and induces a prolonged eightfold increase in the level of 2-AG in the brain while not increasing other acylglycerol or acylethanolamide levels.

Lawrence J. Marnett is in the Department of Biochemistry, Vanderbilt University, School of Medicine, 23rd Avenue at Pierce, Nashville, Tennessee 37232-0146, USA.
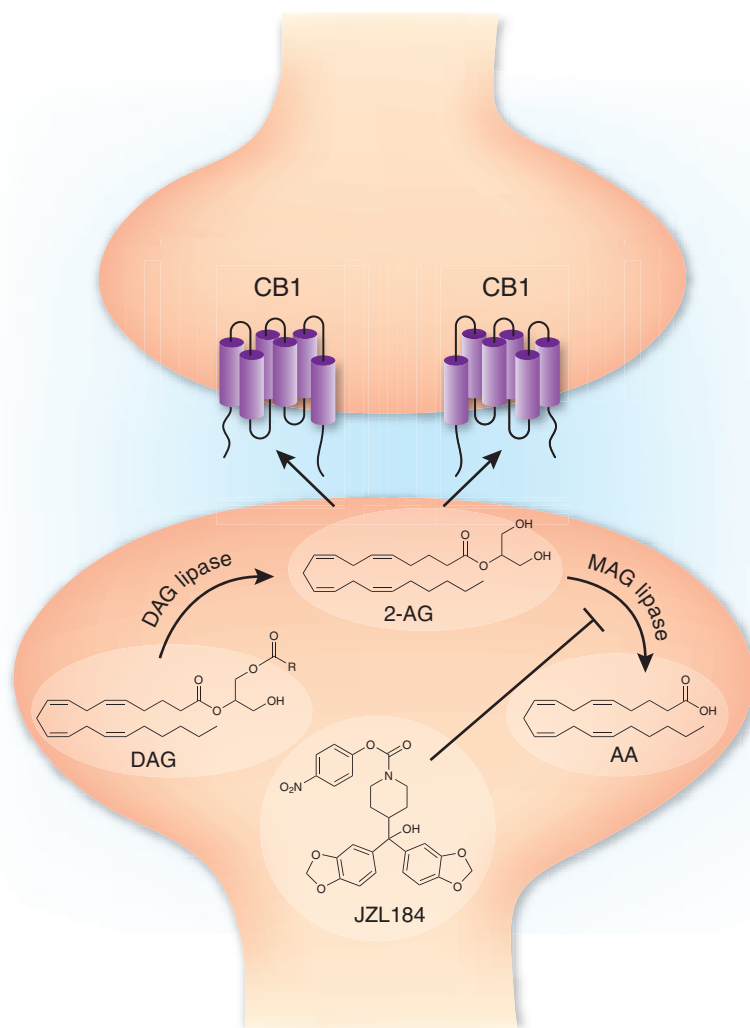*e-mail: larry.marnett@vanderbilt.edu*

Figure 1 2-AG biosynthesis, degradation and signaling through the CB1 receptor. Phospholipase C catalyzes the generation of diacylglycerol (DAG), which is hydrolyzed by DAG lipase to 2-AG. The levels of 2-AG, which is a ligand for cannabinoid receptors (for example, CB1 in the central nervous system), are controlled by hydrolysis by MAGL, the target of JZL184.

Importantly, JZL184 does not interact with other components of the endocannabinoid signaling system (for example, CB1 and CB2 receptors and diacylglycerol lipases α and β) or of arachidonic acid metabolism (for example, phospholipase A_2).

Armed with a powerful and selective inhibitor of MAGL that is active *in vivo*, Long *et al.* probed the effect of pharmacologically increasing 2-AG levels in the brain. Concentrations of JZL184 that inhibit MAGL induced three of the four classic hallmarks of cannabinoid action: analgesia, hypothermia and hypomotility. The fourth cannabinoid effect, catalepsy, was not observed. Each of the cannabinoid effects of JZL184 was blocked by pretreatment of the animals with a CB1 receptor antagonist, which verifies that the effects are mediated by an increase in endocannabinoid levels. Previous experiments have demonstrated that pharmacological inhibitors of AEA hydrolysis exhibit analgesic effects but do not induce hypothermia, hypomotility or catalepsy[5]. The

differential pharmacological effects of FAAH and MAGL inhibitors suggest that, although AEA and 2-AG share analgesic activity, they serve different biological roles even though they work through the same receptor. This points to a highly contextual signaling network for endocannabinoids that depends on the site of generation of 2-AG or AEA, the neurons excited and the stimulus. This selectivity is not replicated by exogenous cannabinoids (such as the active component of marijuana, Δ^9-tetrahydrocannabinol), which stimulate cannabinoid receptors throughout the central nervous system.

Modulators of endocannabinoid tone are active targets for drug development[6]. The fact that JZL184 induces hypothermia and hypomotility as well as analgesia suggests that the clinical development of MAGL lipase inhibitors may be challenging. Nonetheless, the discovery of a potent and selective irreversible MAGL inhibitor that is active *in vivo* represents a significant advance. The growing list of

biological effects of endocannabinoids and the fact that 2-AG is a substrate for conversion to other lipid mediators assures that JZL184 will have immediate and widespread application in a diverse array of biochemical and pharmacological experiments[7,8]. In addition, the pathway of its discovery is a case study in the use of global strategies, such as ABPP, for evaluation of selectivity from the beginning of a drug discovery effort to its successful conclusion.

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A road map of cellular protein homeostasis

Xiaolu L Ang & J Wade Harper

A powerful technology called global protein stability profiling allows rates of protein turnover to be determined for a substantial fraction of the human proteome in a single experiment. This approach sets the stage for systems-level analyses of the dynamics of the mammalian proteome.

Every protein in the cell has a particular lifetime that is reflective of both its intrinsic stability and extrinsic signaling systems that can selectively and temporally alter protein stability rates. Changes in the turnover rates of key proteins underlie developmental and proliferative decisions. As such, the ensemble lifetimes of major signaling proteins define the regulatory state of the cell. Historically, the analysis of protein turnover in mammalian cells has required laborious approaches directed toward one or a small number of proteins within a defined signaling pathway. Thus, a global assessment of how particular regulatory circuits control the rates of protein turnover poses a considerable challenge. Now Yen *et al.* report in a pair of papers[1,2] the development of an innovative strategy, termed global protein stability (GPS) profiling, that allows for massively paralleled analysis of the stability of

Xiaolu L. Ang and J. Wade Harper are in the Department of Pathology, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, Massachusetts 02120, USA.
e-mail: wade_harper@hms.harvard.edu

individual proteins in living cells. This versatile system not only allows a facile measurement of relative protein half-lives but also makes it possible to determine how the stability of a large fraction of the proteome is altered in response to cellular perturbations[1,2].

Previous efforts to understand global proteome dynamics focused on budding yeast, wherein each gene was tagged at its endogenous locus with green fluorescent protein (GFP)[3]. Analysis of this library by flow cytometry revealed how growth conditions altered relative protein abundance and unveiled the importance of biological noise for regulating the responsiveness of protein expression. Analogous approaches in mammalian cells are complicated by genome size and the much more laborious process of targeted homologous recombination. Thus, Yen *et al.*[1] developed an alternative strategy that combines the power of new reporter vectors, fluorescence-activated cell sorting and microarrays in a unique way to create the GPS profiling system.

The crux of the GPS profiling system is a single-copy retroviral–based reporter that

expresses a bicistronic mRNA encoding a red fluorescent protein (RFP) followed by a GFP fusion with a protein of interest—the RFP-IRES-GFP (RIG)-protein fusion (**Fig. 1a**)[1]. Because RFP and GFP fusion proteins are expressed from the same mRNA, the GFP/RFP ratio, as measured by flow cytometry in single cells, provides a measure of protein stability that is independent of the transcriptional regulation of the integrated retrovirus. The GFP/RFP ratio of a series of RIG-degron proteins with varying intrinsic half-lives tracked with their known stability, and the GFP/RFP ratios of RIG-p53 measured in different cell types mirrored the activity of the p53 turnover apparatus in these cells. Thus, the RIG vector can sensitively measure relative turnover rates for individual GFP fusion proteins.

The power of the GPS system is realized by additional technologies that work in tandem to facilitate parallel analysis of thousands of proteins. First, Yen *et al.*[1] generated a library of cells in which each cell stably expresses one of 8,000 human protein-coding open reading frames (ORFs) from the RIG vector (**Fig. 1b**).