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Monoglyceride lipase: Structure and inhibitors

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ABSTRACT

Monoglyceride lipase (MGL), the main enzyme responsible for the hydrolytic deactivation of the endocannabinoid 2-arachidonoyl-*sn*-glycerol (2-AG), is an intracellular serine hydrolase that plays critical roles in many physiological and pathological processes, such as pain, inflammation, neuroprotection and cancer. The crystal structures of MGL that are currently available provide valuable information about how this enzyme might function and interact with site-directed small-molecule inhibitors. On the other hand, its conformational equilibria and the contribution of regulatory cysteine residues present within the substrate-binding pocket or on protein surface remain open issues. Several classes of MGL inhibitors have been developed, from early reversible ones, such as URB602 and pristimerin, to carbamoylating agents that react with the catalytic serine, such as JZL184 and more recent O-hexafluoroisopropyl carbamates. Other inhibitors that modulate MGL activity by interacting with conserved regulatory cysteines act through mechanisms that deserve to be more thoroughly investigated.

1. Introduction

Shortly after the discovery of anandamide in 1992 (Devane et al., 1992), a second endogenous ligand for cannabinoid receptors, 2-arachidonoyl-*sn*-glycerol (2-AG), was identified and quickly recognized as a primary component of endocannabinoid neurotransmission in the central nervous system (CNS) (Mechoulam et al., 1995; Sugiura et al., 1995; Stella et al., 1997). Anandamide and 2-AG activate two G protein-coupled receptors, CB_1 and CB_2 cannabinoid receptors, which are found throughout the mammalian body, but are particularly abundant in the brain (CB_1) and immune cells (CB_2). The endocannabinoids are produced through stimulus-dependent cleavage of membrane phospholipid precursors and are rapidly degraded after their release into the extracellular space. Their on-demand production, their actions explicated near the site of biosynthesis and their rapid deactivation make the endocannabinoids particularly well-suited to mediate short-range neural cellular communication. For example, it has been shown that 2-AG plays a key role in synaptic plasticity and acts as a modulator of neurotransmitter release and neuronal network activity involved in cognition and nociception, among other functions (Hàjos et al., 2000; Izumi and Zorumski, 2012; Higgins et al., 2013). Furthermore, 2-AG-dependent short-range modulation has also been implicated in the development of neurons from neural progenitor cells (Aguado et al., 2005, 2006; Oudin et al., 2011; Gomez et al., 2010; for review, see Galve-Roperh et al., 2013). Similarly, anandamide is responsible for the control of stress-dependent synaptic plasticity in the amygdala (Puente et al., 2011), for the modulation of stress responses and anxiety (Kathuria et al., 2003; Bortolato et al., 2007) and contributes to the control of nociceptive transmission (Clapper et al., 2010).

2. An overview of the endocannabinoid system

2.1. Endocannabinoid biosynthesis

Although anandamide and 2-AG share several chemical and functional properties, they are different in the mechanisms underlying their biosynthesis and the cellular events leading to their deactivation. Three pathways have been identified as responsible for anandamide mobilization (for recent reviews see Ueda et al., 2013 and Piomelli, 2014). An *N*-ayltransferase/phospholipase D-dependent pathway mediates the canonical biosynthetic pathway for anandamide (Di Marzo et al., 1994). On the other hand,

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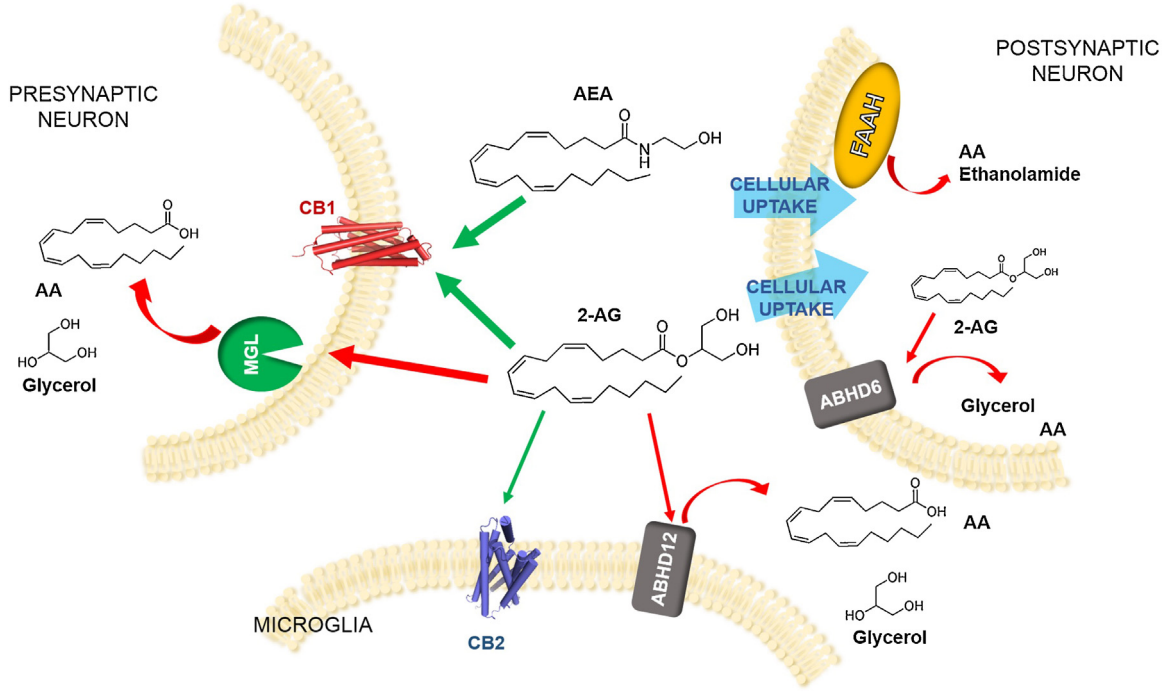

Fig. 1. Schematic overview of 2-AG metabolism.

the route involved in 2-AG production starts from the precursor phosphatidylinositol-4,5-bisphosphate and proceeds through reactions catalyzed by phospholipase C and diacylglycerol lipase (DGL) (Stella et al., 1997; Bisogno et al., 2003; Piomelli et al., 2007).

2.2. Endocannabinoid degradation

Both anandamide and 2-AG are deactivated through a two-steps mechanism, consisting of a selective carrier-based uptake into cells followed by enzymatic hydrolysis. In the CNS, anandamide is hydrolyzed by fatty acid amide hydrolase (FAAH), an amidase-signature enzyme. FAAH hydrolyzes with different efficiencies anandamide, non-cannabinoid fatty acyl ethanolamides such as palmitoylethanolamide (PEA), and also fatty acyl esters, including 2-AG, at least *in vitro*. This apparent promiscuity had led to hypothesize that FAAH might be involved in the hydrolysis of both anandamide and 2-AG; however, this idea has been definitely ruled out by the data. For example, Beltramo and Piomelli found that BTNP ((*E*)-6-(bromo-methylene)-tetrahydro-3-(naphtalen-1-yl)-2*H*-pyran-2-one), an inhibitor of FAAH, does not affect 2-AG hydrolysis at concentrations that block anandamide degradation (Beltramo and Piomelli, 2000). More stringently, Lichtman and coworkers showed that 2-AG hydrolysis is maintained in mutant $FAAH^{-/-}$ mice, and that 2-AG-mediated effects are similar in $FAAH^{+/+}$ and $FAAH^{-/-}$ mice, clearly indicating that an enzyme different from FAAH is responsible for the degradation of 2-AG *in vivo* (Lichtman et al., 2002).

2.3. MGL is the main 2-AG-degrading enzyme

Dinh and coworkers showed that MGL, a serine hydrolase that cleaves 2- and 1-monoglycerides into fatty acids and glycerol (Karlsson et al., 1997), hydrolyzes 2-AG in intact cells (Dinh et al., 2002a). These authors used adenovirus-mediated gene transfer to investigate the role of MGL in 2-AG degradation in brain cortical neurons, and found that MGL overexpression is correlated to attenuation of NMDA/carbachol-induced accumulation of 2-AG, while not affecting 2-AG biosynthesis or anandamide levels. Subsequent experiments using RNAi-mediated silencing (Dinh et al., 2004) and, eventually, deletion by homologous recombination of the *mgll* gene encoding for MGL (Schlosburg et al., 2010), confirmed that MGL plays a key role in the physiological degradation of 2-AG. Dinh and coworkers also determined the anatomical distribution of the enzyme, showing that, in contrast to FAAH, which is broadly distributed in the CNS, MGL is found in discrete areas, including the hippocampus, the cerebellum, the anterodorsal nucleus of thalamus and the cortex (Dinh et al., 2002b). Interestingly, those areas are also characterized by high expression of CB_1 cannabinoid receptors (Hàjos et al., 2000), which is consistent with the role of MGL in terminating the cannabinergic effects of endogenously produced 2-AG near its site of action (Fig. 1). A more detailed characterization of the anatomic distribution of MGL and FAAH showed that the expression of both enzymes matches that of CB_1 receptor, but also that the distribution of MGL is complementary to that of FAAH, and that the two enzymes are situated in distinct neuronal compartments (Gulyas et al., 2004; Dinh et al., 2002b). Immunolabeling experiments localized MGL, similarly to CB_1, to presynaptic nerve terminals of glutamatergic neurons, where the enzyme may contribute to terminate the modulatory effects of 2-AG on excitatory transmission. The presence of MGL in other neuronal subpopulations, including CCK-positive GABA-ergic interneurons, suggests that the lipase may be involved in stopping the effects of 2-AG also in this neuronal population. On the other hand, while the distribution of FAAH overlaps that of CB_1 and MGL at a regional level, its expression is mainly postsynaptic (Dinh et al., 2002b). It is important to point out that the biological functions of MGL are not limited to the deactivation of 2-AG. In addition to mediating the release of fatty acid from monoacylglycerols in adipose tissue (Tornqvist and Belfrage, 1976; Karlsson et al., 1997), MGL also contributes in important ways to the generation of non-esterified arachidonic acid for eicosanoid biosynthesis in a variety of cell types (Bell et al., 1979; Nomura et al., 2011a,b). A role for this enzyme in the control of multiple lipid modulators in cancer cells has also been postulated (Nomura et al., 2010).

While pharmacological and genetic experiments have documented the fundamental role of MGL in 2-AG degradation, there