2-AG + 2 New Players = Forecast for Therapeutic Advances

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In this issue of *Chemistry & Biology*, Blankman et al. [1] identify new enzymes hydrolyzing the endocannabionoid 2-arachidonoyl glycerol (2-AG), increasing the complexity of endocannabinoid signaling in the brain, but also pinpointing additional therapeutic targets.

The promise of cannabinoid-based therapies has been gaining momentum for well over a decade. The anticipation has now reached fever pitch as the first drug targeting the endocannabinoid (eCB) signaling system—the CB1 receptor antagonist rimonabant—has finally reached the public. An article published by Blankman et al. [1] in this issue of *Chemistry & Biology* extends the exciting possibilities for additional cannabinoid-based therapeutic interventions with the identification of new enzymes belonging to the eCB signaling system.

The eCB signaling system consists of the cannabinoid receptors, their endogenous ligands (the eCBs), and the metabolic enzymes that control the availability of those ligands. eCBs are lipid transmitters that are released from membrane precursors by lipases, activate cannabinoid receptors, and are then rapidly inactivated by uptake followed by hydrolysis. The balance between eCB production and inactivation determines the extent of eCB accumulation in tissue and allied cannabinoid receptor activation. Changes in the expression and efficacy of this signaling system have been implicated in a wide range of pathophysiological conditions:

- Cardiovascular/respiratory disorders - hypertension, asthma
- Pain - acute, chronic
- Inflammation - acute, chronic, autoimmune
- Energy metabolism disorders - obesity, anorexia
- Cancer - hematologic, solid
- Gastrointestinal/liver disorders - diarrhea, fibrosis
- Reproductive abnormalities - erectile dysfunction, infertility
- Musculoskeletal disorders - arthritis, osteoporosis
- Eye disease - glaucoma, retinopathy
- Central nervous system disorders - trauma, stroke, multiple sclerosis, Parkinson's disease, Huntington's disease, Tourette's syndrome, amyotrophic lateral sclerosis, epilepsy, Alzheimer's disease, schizophrenia, anxiety, depression, insomnia, nausea/emesis, addiction

The two best-characterized eCBs are anandamide (AEA) and 2-arachidonoyl glycerol (2-AG). The enzyme involved in AEA hydrolysis, fatty acid amide hydrolase (FAAH), has been thoroughly characterized, and its specific inhibitor, URB597, was shown to reduce pain, depression, and anxiety [2, 3], while lacking toxicity and psychoactive effects [4]. There is great hope that additional therapeutics can be developed by selectively targeting 2-AG hydrolysis in vivo, especially in light of the fact that 2-AG is a full and potent agonist at the two principal cannabinoid receptors (CB1 and CB2) [5, 6]. Thus, the palpable prediction is that pharmacological inhibition of 2-AG hydrolysis will provide therapeutic benefit; and monoacylglycerol lipase (MGL) emerged as the strongest candidate for mediating 2-AG hydrolysis in vivo. Indeed, administration of first generation MGL inhibitors leads to selective 2-AG accumulation in discrete brain regions and mimics stress-induced analgesia in a rodent model [7]. However, the molecular mechanism underlying 2-AG hydrolysis appears more complicated than that for AEA. Immunodepletion experiments suggest that MGL accounts for ~50% of the 2-AG hydrolysis activity in brain, implying the existence of additional 2-AG-hydrolyzing enzymes [8]; accordingly, our lab found that microglial cells, the main immunocompetant cells of the brain, hydrolyze 2-AG even in the absence of MGL [9]. This exciting revelation has led us to reason that selective inhibition of a "novel MGL" may induce distinct cannabimimetic effects in vivo, including immune regulation.

Members of the Cravatt lab pioneered the technique of activity-based protein profiling (ABPP), which measures the levels of functionally active enzymes in tissue by directly probing active sites. This versatile technique will likely be instrumental in the characterization and exploitation of a variety of enzyme classes; fortunately for us, the eCB field is among the first to reap its benefits. Using this approach, Blankman et al. report an exhaustive analysis of 2-AG hydrolyzing enzymes in mouse brain, illuminating the physiological relevance of an extensive list of enzymes that may contribute to 2-AG hydrolysis in vivo. While they confirm previous results showing that MGL accounts for ~85% of 2-AG hydrolase activity in mouse brain, they

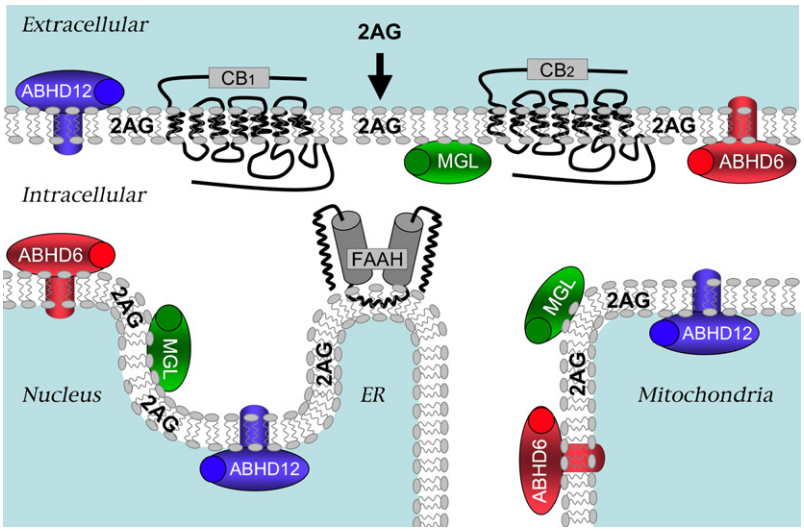

Figure 1. Possible Subcelluar Topology for 2-AG Hydrolyzing Enzymes
Monoacylyglycerol lipase (MGL), ABHD6, and ABHD12 may be located at the plasma membrane, and at membranes of the nuclei and mitochondria. Cannabinoid receptors (CB1 and CB2) are abundant at the plasma membrane, while fatty acid amide hydrolase (FAAH) is abundant at the membrane of the endoplasmic reticulum (ER). The relative importance of MGL, ABHD6, and ABHD12 in controlling 2-arachidonoyl glycerol (2-AG) levels at these membranes remains to be determined. Anandamide is not represented in this model, but is likely also an integral constituent of all these membranes.

also identify the previously uncharacterized serine hydrolases ABHD6 and ABHD12 as new players in the eCB signaling system (one of which is likely to be the "novel MGL" we have been seeking in microglial cells).

Perhaps the most intriguing finding in this report is the observation that MGL, ABHD6, and ABHD12 have distinct membrane topologies. MGL is a cytosolic enzyme that peripherally associates with membranes, ABHD6 is an integral membrane enzyme with its active site facing the cytoplasm, and ABHD12 is an integral membrane enzyme that faces the luminal/extracellular compartments of the cell. This important result should be considered in the context of subcellular locations, for 2-AG hydrolyzing activities are expressed not only on plasma membranes, but also in membranes of the nucleus and mitochondria [9]. Such subcellular repartition suggests that unique 2-AG-hydrolyzing enzymes may regulate distinct pools of 2-AG in intact cells, which should allow for more specific pharmacological modulations of 2-AG levels (Figure 1).

An important consideration when targeting ABHD6 and ABHD12 stems from the unconventional nature of eCB signaling. Unlike classical neurotransmitters, bioactive lipids (including eCBs) tend to be integral mediators within metabolic sequences of enzymatically catalyzed signaling cascades, with each successive molecule being responsible for a distinctive biological response, and each enzyme exhibiting a unique profile of substrate selectivity. Therefore, inhibiting MGL, ABHD6, and ABHD12 may have secondary effects beyond simply increasing 2-AG levels. Such effects are bound to be different depending on which 2-AG hydrolyzing enzyme is inhibited, and targeting one enzyme may be more beneficial than another.

The potential importance of ABHD6 and ABHD12 compared to the highly active MGL should not be underestimated, as their respective roles may differ depending on cell type and changes in expression due to, for example, pathological conditions. ABHD6 and ABHD12 will likely prove to be attractive new therapeutic targets, occupying unique niches within the eCB system. In support of such a notion, we suggest considering the critical importance of the sparse CB1 receptors expressed by glutamatergic neurons, which surprisingly mediate most of the psychotropic effects of cannabinoid compounds, in contrast to the as yet unclear role of the abundant CB1 receptors expressed by GABAergic neurons [10]. With 2-AG emerging as the predominant mediator of eCB-dependent synaptic plasticity [11], pharmacological and genetic tools targeting ABHD6 and ABHD12 will provide neuroscientists with valuable new strategies for studying and manipulating synaptic physiology. This could have important implications for the fields of cognition, learning, memory, and development. As our appreciation of the fundamental, ubiquitous, and evolutionarily ancient nature of the eCB system continues to grow, so too will the list of disciplines that could benefit from tools to manipulate 2-AG signaling.

It is gratifying to see that modern science is now able to exploit the medicinal properties of marijuana (which people around the world used as medicine for thousands of years) by taking advantage of our newly acquired (and still emerging) knowledge of its mechanisms of action. Furthermore, as these mechanisms continue to reveal themselves, it becomes easier to separate the medicinal benefits from the drug of abuse aspects that have stigmatized marijuana. However, our ability to fashion specialized eCB-based therapies depends on a comprehensive understanding of this complicated and pervasive signaling system. With their contribution, Blankman et al. have provided an integral piece of the puzzle by identifying two important new players in this fascinating signaling system.

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Fatty Pain Cures

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In this issue, Alvin King, Daniele Piomelli, and colleagues publish another interesting paper on inhibition of monoacylglycerol lipase (MGL). MGL is a hot target for antinociceptive agents, being the chief degrading enzyme of the endocannabinoid 2-arachidonoylglycerol [1].

"The problem of pain" [2] has troubled everybody, often to the verge of despair. Equally troublesome and despairing have been efforts at developing analgesics. Pathways were discovered that simply must have something to do with sensing, transmitting, and realizing pain, and inhibitors were found that worked ever so well in the usual animal models of antinociception—but in humans, they apparently failed to cause or display distinct effects. In humans at least, pain is a very subjective state of perception, and that is probably why linear extrapolations from molecular to clinical effects are rarely possible. Perhaps the same biochemical events will be interpreted differently by the mind when it comes to states of consciousness like pain? To, as it were, chemically detach a patient from feeling his pain, without impairing his emotional and intellectual capacities, remains a major challenge with very likely no satisfactory solution.

Listing all known molecular targets of approved drugs [3], we identified eight whose stimulation or blockade are thought to lead to analgesia or antinociception, not counting the targets of neuroleptics and tranquilizers that have an analgetic by-effect. Against this background, new molecular targets that hold the promise of being relevant for nociception are always welcome. In this context, nociception or hyperalgesia means that even slight touches or pressures (e.g., caused by swellings or inflammation) induce pain, as opposed to pain caused by a fracture or hard blow. The endocannabinoid—or rather eicosanoid—system that has been discovered during the past years is strongly involved in basic sensory physiology including nociception.

Presently, the endocannabinoid system [4, 5] is known to consist mainly of: (1) two receptors of the G protein coupled receptor family, CB1 and CB2; (2) endogeneous ligands that are derived from arachidonic acid, like anandamide and 2-arachidonoyl glycerol (2-AG); (3) a transporter of anandamide that has escaped thorough characterization so far; and (4) hydrolases that catalyze the biosynthesis and inactivation of the ligands. 2-AG is inactivated by two or more monoacylglycerol lipases (MGL, MAGL), while anandamide is hydrolyzed by fatty-acid amide hydrolase (FAAH) and N-acylethanolamine acid amidase (NAAA).

CB receptors are supposed to be more numerous in the CNS than dopamine receptors, and they were also found in other body tissues. Endocannabinoid signaling, which is of the short-range short-term type, is strongly involved in antinociception, anxiolytic action, cell proliferation, reproduction, memory processes, and modulation of feeding [3].

Due to its abuse, it has long been known that cannabis has analgesic effects. At the moment, the following modulators of the endocannabinoid system are thought to lead to analgesic action: agonists at CB1 and/or CB2 receptors, inhibitors of FAAH, and inhibitors of MGL. The latter two interferences would work indirectly by increasing the amount of endocannabinoids.

Cause-and-effect is anything but clear with endocannabinoids. Different literature reports have shown, for instance, the CB2 receptor and 2-AG to be strongly involved in stimulation and in attenuation of inflammation and immune responses [6]. Apart from the consideration on nondeterminedness of psychopharmaceutical action, the intertwining of the CB and other pathways need to be taken