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So FAAH, so good, maybe

By Lev Osherovich, Senior Writer

A paper in *Nature Chemical Biology* describes the potent analgesic effect of a class of organophosphorus compounds that enhances cannabinoid receptor activity.[1] Unlike pain treatments based on agonizing cannabinoid receptors with compounds such as cannabis-derived tetrahydrocannabinol, these organophosphorous compounds inhibit fatty acid amide hydrolase and monoacylglycerol lipase, two enzymes responsible for degrading the major endocannabinoids anandamide and 2-arachidonylglycerol.

Academics and companies are still hashing out which of the two enzymes is the better target for therapeutics, and opinions are also mixed on the toxicity and potential side effects of the organophosphorous compounds described in the paper.

Fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) control the levels of endocannabinoids, neurotransmitters that are the natural ligands of cannabinoid receptors. In the CNS, endocannabinoids turn on cannabinoid receptor 1 (CB_1) but are rapidly inactivated by both FAAH and MAGL. In the peripheral nervous system, a second cannabinoid receptor, CB_2, is primarily agonized by 2-arachidonylglycerol (2-AG).[2] (*See* **Figure 1, "Targeting cannabinoid receptors, monoacylglycerol lipase and fatty acid amide hydrolase."**)

Earlier studies hinted that organophosphorus compounds, such as the chemical warfare agent sarin, hit a broad range of hydrolases and lipases in addition to their primary target, acetylcholinesterase (AChE). The team on the *Nature Chemical Biology* paper, led by John Casida, professor of toxicology at the **University of California, Berkeley**, suspected that these secondary targets could include FAAH and MAGL.

To test this hunch, the team used a group of organophosphorus compounds that preferentially inhibit FAAH and MAGL while leaving AChE alone, and then they looked at the resulting endocannabinoid-related behavior in mice. The best of these compounds, isopropyl dodecylfluorophosphonate (IDFP), profoundly affected the behavior of exposed mice.

"These mice had much more potent cannabinoid effects" than those caused by tetrahydrocannabinol (THC), said graduate student Daniel Nomura, who was the lead author of the study. The mice developed all the hallmarks of cannabis intoxication, including the therapeutically useful analgesia and increased appetite, but also had hypothermia, catalepsy and reduced mobility.

To prove these effects were due to high endocannabinoid activity rather than nonspecific toxicity, Nomura repeated the experiments in mice lacking CB_1. Such mice cannot respond to endocannabinoids and were also unaffected by IDFP.

The problem was that IDFP's highly reactive organophosphate group had off-target effects. After about a week, the mice developed signs of neuropathy and had to be euthanized, Nomura told *SciBX*.

Indeed, these organophosphorus compounds are the most potent inhibitors of FAAH and MAGL ever reported. Industry researchers contacted by *SciBX* agreed that the paper provides a new chemical scaffold for inhibiting the two enzymes, but they had mixed opinions on whether the scaffold could ever yield good therapeutic candidates.

Nomura noted the study does shed light on how organophosphate toxicity may affect the nervous system, potentially aiding in the design of therapeutics against nerve agents and toxic pesticides.

"One of the main points is that some of the secondary effects of organophosphorus agents may be mediated through cannabinoid signaling," Nomura said. "The bioactivated metabolite of the insecticide chlorpyrifos is one of the compounds."

FAAH inhibitors

Fabien Vincent, senior scientist at the Renovis Inc. subsidiary of **Evotec AG**, said an organophosphorus scaffold for future drug development "adds more to the landscape" for the next generation of FAAH inhibitors. He also thinks the new compounds compare favorably to the carbamates, ureas and ketooxazole FAAH inhibitors currently in development.

The challenge, Vincent said, is to tone down the reactivity and improve the selectivity of the new compounds. "It's a new potential class of inhibitors, but it will be very tricky to make it into a drug," he told *SciBX*.

Evotec gained an FAAH inhibitor program when it completed its acquisition of Renovis in March.

One problem with the Berkeley group's compounds, said Vincent, is that the highly reactive organophosphate group makes them covalently modify the target enzyme's active sites.

"Inhibitors that covalently modify targets are considered a bad thing" by pharma companies, he said. This is because covalent modification of enzymes is irreversible and can lead to undesired side effects.

Vincent said that unlike other FAAH inhibitors, Evotec's compounds "are not based on a highly reactive scaffold" and thus are less likely to irreversibly inactivate other enzymes. He would not disclose the company's specific scaffolds.

Daniele Piomelli, professor of pharmacology at the **University of California, Irvine**, also thinks that despite improvements over previous compounds, the molecules published in the study are insufficiently specific for use as therapeutics.

"Organophosphorus agents are toxins," said Piomelli. Although the compounds hit fewer off-pathway targets than expected for such reac-

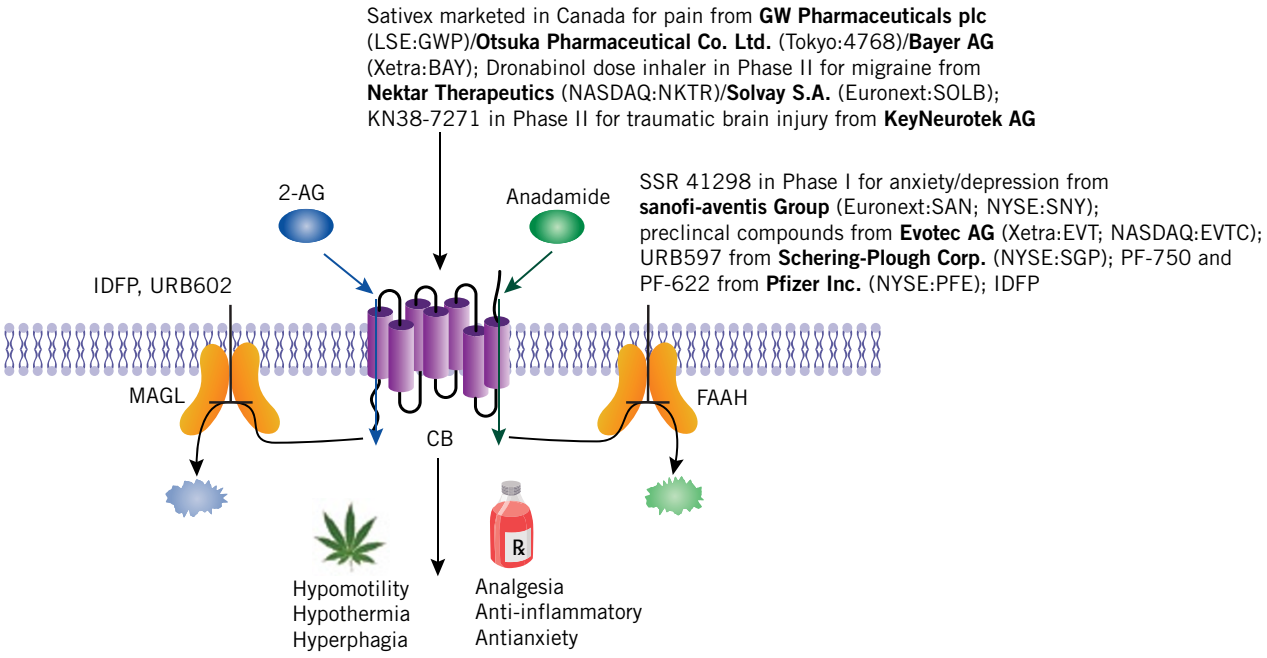

Figure 1. Targeting cannabinoid receptors, monoacylglycerol lipase and fatty acid amide hydrolase. Compounds that block monoacylglycerol lipase (MAGL) and/or fatty acid amide hydrolase (FAAH) could lead to treatments with minimal side effects associated with cannabinoid (CB) receptor activation. Endocannabinoids such as anandamide and 2-arachidonoylglycerol (2-AG) bind to the CB receptor, promoting both desirable effects (Rx), such as analgesia or anti-inflammation, and undesirable effects (hemp leaf). 2-AG is degraded by MAGL, and anandamide is degraded by FAAH. Nomura *et al.*[1] found that organophosphorus agents such as isopropyl dodecylfluorophosphonate (IDFP) block both FAAH and MAGL. This prevents the degradation of anandamide and 2-AG and results in constitutive activation of CB receptors. Researchers are still hashing out how to obtain the benefits of CB receptor activation without the side effects. The figure shows selected CB receptor agonists in the clinic and endocannabinoid hydrolase inhibitors in development for pain, inflammatory or psychiatric indications.

tive molecules, he suspects that the "endocannabinoid component of these drugs doesn't explain the toxicity."

Nomura believes that the long-term toxicity of the organophosphorus compounds in the study was probably due to off-pathway inhibition of another enzyme called neuropathy target esterase.

Piomelli suggested that THC derivatives remain a safer choice despite their own shortcomings.

MAGL mystery

Though the organophosphorus compounds singled out by Casida and colleagues are not yet suitable therapeutics, Nomura told *SciBX* they are the first effective MAGL inhibitors, opening the door to studying the effects of this enzyme on pain.

According to Piomelli, figuring out what happens when MAGL is inhibited is the "$64,000 question" in endocannabinoid research. 2-AG is thought to be the predominant ligand for cannabinoid receptors in both the central and peripheral nervous systems, which have "about 100–200 times more 2-AG than anandamide," he said.

Iris Alroy, VP of discovery at **Pharmos Corp.**, told *SciBX* that boosting 2-AG and CB_2 activation in the peripheral nervous system potentially could treat pain without eliciting the side effects of CB_1 activation in the CNS. Pharmos is developing agonists and antagonists of CB_2.

Alroy cited evidence that in mouse models of neuropathic pain, FAAH inhibition "does not result in analgesic activity," which suggests MAGL may be a better target.

Until now, the lack of good inhibitors and a MAGL knockout mouse has made it hard to assess 2-AG's role in endocannabinoid signaling. Piomelli's previous studies yielded URB602, which he described as a "very weak inhibitor." However, URB602 enhanced stress-induced analgesia in a rodent model for pain.[3,4]

Piomelli was a cofounder and CSO of pain company Kadmus Biopharmaceuticals Inc., which sold its assets to Organon BioSciences N.V. (now part of **Schering-Plough Corp.**) in 2007. Schering-Plough would not disclose the development status of Kadmus' FAAH inhibitor, URB597.

Nomura said the next step will be to develop more MAGL-selective versions of the compounds in this study. This work will be done in the laboratory of the study's coauthor, Benjamin Cravatt, professor of cell biology and chemistry at **The Scripps Research Institute**. Cravatt's team has previously worked with **Pfizer Inc.** to develop PF-750 and PF-622, two FAAH inhibitors based on piperidine/piperazine urea scaffolds.

Although PF-750 and PF-622 are also irreversible inhibitors of FAAH, they do not hit MAGL and do not produce as profound an effect as observed with the organophosphorus compounds.[5] Thus, the

added potency of new compounds may be due to the inhibition of MAGL as well as FAAH.

The findings are not patented, according to Nomura.

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COMPANIES AND INSTITUTIONS MENTIONED

Evotec AG, (Xetra:EVT; NASDAQ:EVTC), Hamburg, Germany
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Pharmos Corp. (NASDAQ:PARS), Iselin, N.J.
Schering-Plough Corp. (NYSE:SGP), Kenilworth, N.J.
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