FLATtening pain

By Lev Osherovich, Senior Writer

Italian and Californian researchers have discovered a protein called FAAH-like anandamide transporter that helps inactivate the endocannabinoid signaling molecule anandamide, a naturally occurring analgesic.¹ The team has created a small molecule antagonist of the transporter protein that could be useful for treating pain.

Anandamide is a fatty acid amide that activates cannabinoid receptors at synapses in the brain. Pharms have sought to mimic the analgesic effects of anandamide with centrally acting cannabinoid receptor agonists, but such compounds—for example, tetrahydrocannabinol—elicit cognitive side effects.²

Thus, companies have been looking for targets upstream of cannabinoid receptors, such as enzymes involved in transporting and inactivating endocannabinoids. The rationale is to increase the levels of endocannabinoids by blocking either their reuptake from the synaptic cleft or their subsequent degradation.

Companies have had success with the latter approach—at least four pain compounds in development inhibit fatty acid amide hydrolase (FAAH), a serine hydrolase that degrades a variety of endocannabinoids. Efforts to block cannabinoid reuptake have not borne fruit, as the molecular players involved in the transport of endocannabinoids from the synaptic cleft were unknown.

Now, a joint team from the **University of California, Irvine** and the **Italian Institute of Technology's** Drug Discovery and Development (D3) research unit has identified FAAH-like anandamide transporter (FLAT) as a splicing variant of FAAH that has a distinct role in endocannabinoid signaling. FLAT binds to anandamide but lacks FAAH's catalytic activity. Instead of degrading anandamide, FLAT transports the endocannabinoid to FAAH's site of action in membrane-bound compartments in neurons.

"We believe that FLAT acts as a shuttle to transfer anandamide from the plasma membrane to intracellular membranes, where it becomes inactivated by FAAH," said team leader Daniele Piomelli, professor of pharmacology at UC Irvine and director of D3. Thus, blocking FLAT could increase anandamide's analgesic effects by preventing the endocannabinoid from being degraded by FAAH.

Searching FAAH and wide

Piomelli's team came across a transcript encoding FLAT in a study of rat brain mRNA. Compared with full-length FAAH, FLAT lacks 67 amino acids near the N-terminus, corresponding to a region of FAAH needed

for membrane localization.

The team found that recombinant FLAT could bind to anandamide *in vitro* but, unlike full-length FAAH, did not degrade anandamide. Nonetheless, cultured cells expressing transgenic FLAT accumulated anandamide in their cytoplasm at higher levels than nontransgenic controls.

These findings suggested FLAT and FAAH have distinct roles in transporting and degrading anandamide, respectively.

Thus, Piomelli suspected FLAT, which was not confined to the surface of membranes, could shuttle anandamide between different membrane-bound cell compartments. Indeed, the effects of FLAT expression on intracellular anandamide levels were abolished by treating cells with broad-spectrum endocannabinoid transport inhibitors.

While Piomelli's team at UC Irvine characterized the biology of FLAT, his group at D3 identified ARN272, a high-affinity, selective FLAT antagonist. In a mouse model of acute pain, ARN272 prevented pain-related behavior compared with vehicle controls.

Results were published in *Nature Neuroscience*.

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—Daniele Piomelli,
University of California, Irvine

Fix a FLAT

Academic and industry researchers in the FAAH space said ARN272 could become a promising drug candidate but that additional work is needed to unravel FLAT's role in endocannabinoid signaling and to directly compare the effects of FAAH and FLAT inhibition.

"The existence of anandamide transporters has been discussed in the literature for a long

time," but evidence for a specific protein involved in that process has been missing until now, said Badia Ferzaz, operations director at **Sanofi**. Sanofi's SSR411298 FAAH antagonist is in Phase IIa testing for cancer-associated pain.

"It's still controversial whether there really is a transport mechanism" for anandamide and other endocannabinoids, said Daniel Nomura, assistant professor of nutritional sciences and toxicology at the **University of California, Berkeley**. "I think there has to be some kind of transporter or binding protein because these compounds are very insoluble but still somehow traverse the synaptic cleft" to be degraded by FAAH.

Nomura said previous mouse knockout studies by Piomelli's team suggested animals lacking the *FAAH* gene could still transport anandamide,³ although Piomelli noted that *FAAH* knockouts have diminished anandamide transport compared with wild-type controls.

But because knocking out *FAAH* should also eliminate FLAT expression, Nomura thinks Piomelli's previous work hints that other players besides FLAT contribute to anandamide uptake.

The real question is how the analgesic effects of blocking FLAT stack up against blocking FAAH, which will require head-to-head studies of ARN272 and FAAH inhibitors.

"It's difficult to know how [ARN272] lines up versus FAAH inhibitors in the absence of a positive control," said Stephen Pawsey, development director and CMO of **Vernalis plc**.

Vernalis' V158866 FAAH inhibitor has completed Phase I testing.

Piomelli cited preliminary, unpublished data from his lab suggesting ARN272 is more effective than FAAH inhibitors in rodent models of acute pain. He said ARN272 needs further optimization but could be the starting point for a clinical candidate.

Whether to pursue FAAH or FLAT also may depend on the specific pain indication.

Pawsey said that both the clinical experience with cannabinoids and preclinical studies have suggested that raising anandamide levels can alleviate neuropathic pain, so targeting either FAAH or FLAT could be useful in that setting.

On the other hand, Ferzaz said other endocannabinoids besides anandamide can help decrease inflammatory pain. These other endocannabinoids, including palmitoylethanolamide (PEA) and oleoylethanolamine (OEA), are degraded by FAAH.

Because blocking FAAH would increase levels of multiple endocannabinoids, whereas blocking FLAT would only affect anandamide levels, Ferzaz and Pawsey favor targeting FAAH for inflammatory pain indications.

Pawsey said Vernalis is selecting an indication for a Phase II proof-of-concept trial of V158866. The study is expected to start next year.

Other FAAH inhibitors in the clinic for pain are IPI-940, from **Infinity Pharmaceuticals Inc.**, **Purdue Pharma L.P.** and **Mundipharma International Ltd.**, which completed Phase I testing, and **Ironwood**

Pharmaceuticals Inc.'s IW-6118, which completed Phase II testing for molar extraction pain. Last year, **Pfizer Inc.**'s FAAH inhibitor failed in Phase II testing for osteoarthritic pain.

Piomelli has patented his findings. ARN272 is available for licensing from D3.

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COMPANIES AND INSTITUTIONS MENTIONED

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