New inhibitors of elusive enzymes promise to be valuable scientific tools

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(Phys.org)—Scientists at The Scripps Research Institute (TSRI) have discovered the first selective inhibitors of an important set of enzymes. The new inhibitors, and chemical probes based on them, now can be used to study the functions of enzymes known as diacylglycerol lipases (DAGL), their products, and the pathways they regulate. Early tests in mouse macrophages suggest that DAGL-inhibiting compounds might also have therapeutic uses, for they suppress the production of a pro-inflammatory molecule that has been implicated in rheumatoid arthritis and related conditions.

"We've developed the first set of chemical probes that effectively allows one to study these DAGL enzymes in living cell and animal models," said Benjamin F. Cravatt, chairman of the Department of Chemical Physiology, professor in the Dorris Neuroscience Center and member of the Skaggs Institute for Chemical Biology at TSRI. Cravatt and his laboratory conducted the new study, published in the current issue of the journal *Nature Chemical Biology*.

Important But Poorly Understood

DAGL enzymes have been of interest mainly because of their role in making 2-AG (2-Arachidonoylglycerol), an important cannabinoid that is naturally produced in humans and other mammals. Cannabinoids are named for Cannabis (marijuana) plants, because they stimulate the same cellular receptors that are hit by marijuana's active ingredients. Drugs

that can enhance 2-AG's signaling in the nervous system are being developed as treatments for pain, depression and anxiety.

But 2-AG exists in various tissues throughout the body, and on the whole, its functions are not well understood. Until now researchers have lacked enzyme inhibitors that can usefully probe those functions by selectively shutting off 2-AG's production. "Existing DAGL inhibitors block many other enzymes, are not very potent, and do a poor job of getting into cells," Cravatt said. "There has been a need for better chemical tools in this area."

Cravatt's laboratory had previously developed a set of compounds that act as potent inhibitors of serine hydrolases—the broad enzyme family to which DAGL enzymes belong. In the new study, Cravatt's team, including first author Ken Hsu, a Hewitt Foundation postdoctoral researcher in the Cravatt laboratory, screened a library of these compounds for specific activity as DAGL inhibitors.

A Big Improvement

After finding a promising lead compound, Hsu and his colleagues chemically optimized it to obtain KT109 and KT172. The former selectively inhibits DAGLβ, the main enzymatic producer of 2-AG outside the nervous system. KT172 inhibits both DAGLβ and DAGLα, which is principally responsible for making 2-AG within the nervous system.

In a big improvement over previously described DAGL inhibitors, KT109 and KT172 are highly selective (i.e., they do not block many other, non-DAGL enzymes) and active in cells and animals. By analyzing the structures of their initial DAGL inhibitors, the team was also able to devise a new DAGL-tailored activity-based probe that binds to the active site of DAGLs and fluorescently labels these low-abundance and

difficult-to-detect enzymes in cell or tissue samples. "Without the DAGL-specific probe, we would have found it very difficult to develop, optimize and confirm target engagement for our DAGL inhibitors," Hsu said.

In neuron-like mouse cells, human prostate cancer cells, and mouse liver cells and macrophages (a type of immune cell that is frequently involved in inflammatory conditions), the DAGL inhibitors were able to inactivate DAGLβ activity. "At the optimal doses used, we were able to achieve selective and near-complete inhibition of the enzyme," said Hsu. In these cell and animal studies, the inhibitors also reduced levels of 2-AG as well as arachidonic acid, another bioactive lipid that DAGL enzymes can regulate.

New Questions

2-AG is known to have an anti-inflammatory effect when it activates cannabinoid receptors on macrophages. Thus, one might expect that knocking down 2-AG production with a DAGL inhibitor would have a pro-inflammatory effect. Instead, Hsu, Cravatt and their colleagues found that blocking DAGL in mouse macrophages that had been stimulated with pro-inflammatory agents markedly lowered their secretion of TNFα, a major inflammatory signaling molecule.

Blocking DAGL has potential effects on multiple lipid signaling pathways in cells, and the researchers aren't yet certain which of these effects explains the surprising suppression of TNFα. "The effect is dependent on DAGLβ, though, because we see the same result in DAGLβ knockout mice," said Hsu. Cravatt added that their observations of the unexpected DAGL-inhibition effects in mouse macrophages could be due to the suppression of pro-inflammatory eicosanoids that derive from downstream metabolites regulated by DAGLβ.

TNFα is a key instigator of the inflammation seen in rheumatoid arthritis, and antibodies directed against TNFα are now front-line therapies for the condition. "What we've done so far is just early-stage cell biology, but conceivably the further optimization of our DAGL inhibitors could result in a new type of anti-inflammatory drug that also works against arthritis and related conditions," Cravatt said.

Cravatt and his team are now studying the pathways through which the new inhibitors have this anti-inflammatory effect. They also plan to develop new inhibitors that will selectively block DAGLα and 2-AG production in the central nervous system.

More information: "DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses," *Nature Chemical Biology*.

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