Study solves structure of 'salvia receptor', reveals how salvinorin A interacts with it

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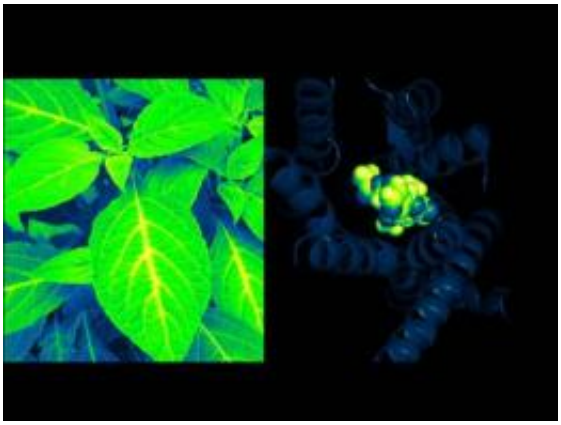

On the left panel is a *Salvia divinorum* plant. On the right panel, location of drug salvinorin A in the kappa opioid receptor. Credit: Roth lab, UNC-Chapel Hill.

At the molecular level, drugs like salvinorin A (the active ingredient of the hallucinogenic plant *Salvia divinorum*) work by activating specific proteins, known as receptors, in the brain and body. Salvinorin A, the most potent naturally occurring hallucinogen, is unusual in that it interacts with only one receptor in the human brain — the kappa opioid receptor (KOR). Scientists know of four distinct types of opioid receptors, but until now the structure of the 'salvia receptor', and the details about how salvinorin A and other drugs interact with it, was a mystery.

Scientists have for the first time determined the three-dimensional

atomic structure of a human opioid receptor, a molecule on the surface of brain cells that binds to opioids and is centrally involved in pleasure, pain, addiction, depression, psychosis, and related conditions. Dozens of legal and illegal drugs, from heroin to hospital anesthetics, work by targeting these receptors. The detailed atomic structure information paves the way for the design of safer and more effective opioid drugs.

"This finding is going to have a major impact on understanding the fundamental principles of opioid receptor recognition and evolution," said Raymond Stevens, PhD, a professor at The Scripps Research Institute. Stevens is the senior author of the new study, which appears online in the journal *Nature* on March 21, 2012.

A Symphony of Activity

Opioid receptor subtypes in the human brain work together in a symphony of activity that is still not fully understood. The "mu" opioid receptors mediate feelings of pleasure and pain-relief; they are the prime targets of the body's own endorphin neurotransmitters as well as heroin, morphine, and most other opioid drugs. By contrast, "kappa" opioid receptors are bound by neurotransmitters known as dynorphins, and when activated can depress mood and produce dissociative, psychedelic experiences. The plant Salvia divinorum, which was originally cultivated by Mesoamerican societies for religious ceremonies and is now used widely as a recreational drug, has an active ingredient, Salvinorin A, that binds selectively and with high affinity to kappa opioid receptors.

"We don't know why kappa receptors evolved, but we know that they have been around for a long time in evolutionary terms; even frogs have them," said Bryan Roth, a professor of pharmacology and an opioid receptor expert at the University of North Carolina, whose group teamed with the Stevens lab for the new study.

If their psychedelic and mood-darkening effects could be avoided somehow, kappa opioid receptor activators, or "agonists," could be very useful medically. In animal studies, they act as mild and non-addicting pain-relievers, weaken the addictive effects of other drugs, and reduce irritable bowel signs. "Antagonist" compounds that block kappa opioid receptor activity also show promise as treatments for depression, anxiety, and other psychiatric conditions. Even the psychedelic effects associated with kappa receptor activation could be useful in providing insights into perception and consciousness. "This is a receptor that is important for how we see reality," said Roth.

In Pursuit of Missing Information

Not knowing the structural details of the kappa opioid receptor has made it hard for scientists to understand how the receptor works naturally and to design drugs that target it in the right ways. There are currently no FDA-approved drugs that bind selectively to kappa opioid receptors, either as agonists or antagonists.

To get these structural details, Scripps Research graduate student Huixian Wu, who was first author of the paper, first produced kappa opioid receptors in insect cells and used special techniques to keep the fragile receptor molecules stable in a single conformation. Working with Scripps Research Assistant Professor Vadim Cherezov, PhD, Wu was then able to crystallize and collect x-ray data that eventually led to the structure. An essential part of this stabilizing process involved the attachment of a suitable "ligand," a pharmacological molecule that binds the receptor tightly. "We tried a lot of different ligands, and Bryan suggested that we try JDTic, an experimental kappa opioid receptor antagonist," said Stevens. "It worked well in stabilizing the receptor, and once we discovered that, everything else quickly fell into place."

By X-raying such a crystallized protein complex from various angles,

researchers can calculate a detailed three-dimensional model of its atomic structure. In this case, the X-ray data yielded a model with a resolution of about 2.9 angstroms, or 290 trillionths of a meter. The Stevens and Roth labs then designed and performed site-specific mutagenesis analyses in which they altered various residues of the receptor to better understand how its structure related to its function. Scripps Research Assistant Professor Seva Katrich, PhD, together with colleagues at Research Triangle Institute and Virginia Commonwealth University, then performed molecular modeling and docking analyses to further the understanding of the receptor-ligand interactions.

"This receptor's binding pocket is much bigger and deeper than any other we've studied; that may explain why so many different types of ligands bind to this receptor. A lot of work remains, though, in understanding opioid selectivity. As with the other receptor structures, this is just the beginning and we will see follow up studies take the understanding even further," said Stevens. He and his colleagues used their structural model to learn new details about the kappa receptor binding characteristics of Salvinorin A plus an array of experimental drugs.

With the publication of the receptor structure, scientists and drug companies also will be able to use the data to improve existing kappa-targeting compounds and design entirely new ones. "I can tell you, from e-mails and other conversations I've had recently, that people in the pharma industry are very eager to see this structural data," said Roth.

Provided by University of North Carolina School of Medicine