At the 2012 ICRS conference

Mechoulam's To-Do List for Researchers: CBD, the CB2 Receptor, and 'F-Triple-A's'

By O'Shaughnessy's News Service

Raphael Mechoulam, professor of Medicinal Chemistry and Natural Products at the Hebrew University of Jerusalem, began "reading on cannabis and planning some limited amount of work on it" in 1962.

The work turned out to be limitless.

Over the course of 50 years Mechoulam has made and participated in a remarkable series of Cannabis-related achievements. He and his colleagues isolated and elucidated the chemical structure of THC, CBD, and many other plant cannabinoids (a term Mechoulam himself coined). They did the same for the endogenous cannabinoids, anandamide and 2-AG. They figured out the steps by which these compounds are synthesized in the body and exert their effects by activating receptors. Their accomplishments —like all scientific advances— have extended the research agenda.

"Planning Research for the Next Half a Century" was the title of Mechoulam's talk at the International Cannabinoid Research Society meeting in Freiburg, Germany in the summer of 2012.

If Mechoulam's speculation about the role of FAAAs can be substantiated, it will represent an advance in scientific understanding as significant as any he has contributed to in the past.

Mechoulam pointed to three areas of investigation likely to yield important medical discoveries: cannabidiol (CBD), the CB2 receptor system, and fatty acids bound to amino acids (FAAAs, pronounced "F-triple-A's"). FAAAs are signalling molecules abundant in the brain. They are found in clusters that include their precursor molecules and their derivatives. Anandamide and 2-AG are among the few FAAAs that have been studied to date.

If Mechoulam's speculation about the role played by FAAA clusters in the brain can be substantiated, it will represent an advance in scientific understanding as significant as any he has contributed to in the past.

• Cannabidiol

CBD is a non-psychoactive compound with no known adverse effects. It is a potent anti-inflammatory, a quality recognized by physicians in ancient Greece and Rome, Mechoulam said, where the available Cannabis was of the hemp type (with virtually no THC).

Mechoulam studied the anti-inflammatory effects of CBD in collaboration with his Hebrew University colleague Ruth Gallily, whose *in vitro* experiments showed that increasing doses of CBD cause cells cultured from the lining of arthritic joints to diminish production of pro-inflammatory compounds.

Experiments with mice by Gallily and Mark Feldmann of Imperial College, London, confirmed that CBD alleviates symptoms of rheumatoid arthritis such as swelling. They also confirmed that CBD has a biphasic effect — there is an optimal dose, below which and above which it is less potent.

"So I hope somebody will work with CBD or one of its derivatives as an anti-rheumatoid arthritis agent," said Mechoulam, hopefully.

Given that rheumatoid arthritis is an autoimmune disorder, Mechoulam decided to test the ability of CBD to counter the symptoms of diabetes type-1, another disease in which the immune system mistakes the body's own cells for pathogens. He described a study involving a strain of mice that develop diabetes type-1 at about 14 weeks. Treatment with CBD resulted in only 30 percent of the mice becoming diabetic (instead of 80-to-100 percent). Damage to the pancreas was reduced proportionally, with more than 70 percent of the hormone-producing islets remaining intact in the CBD-treated mice.

Raphael Mechoulam photo by Zach Klein

Unfortunately, Mechoulam said, clinical trials of this very promising treatment for diabetes type-1 cannot be conducted until funding becomes available.

"Chances are we'll see similar results with CBD in psoriasis and many other autoimmune diseases," he predicted, when the research can be carried out.

Elucidating the mechanisms by which CBD works is a big item on Mechoulam's to-do list. Although CBD has little binding affinity with the two known cannabinoid receptors, it confers a therapeutic effect through various receptor-independent channels and by directly activating or antagonizing several non-cannabinoid receptors.

Diabetes type-2 and obesity are characterized by chronic, low-grade inflammation. White blood cells build up in visceral adipose tissue (VAT), leading to insulin resistance and other problems. Citing several recent papers, Mechoulam suggested that CBD might play a protective role in diabetes type-2 via a receptor (PPAR-γ) that regulates fat-cell development.

• The CB2 receptor

We mammals have a sophisticated immune system that guards against foreign proteins and reduces damage they cause. "We must have an analogous system protecting against non-protein attack," Mechoulam stated.

The CB2 receptor may play a central role in such a protective system. "Endocannabinoids and endocannabinoid-like molecules acting through the CB2 receptor have been reported to affect a large number of pathological conditions," Mechoulam said.

Stimulation of CB2 "lowers pro-inflammatory cytokines [chemical messengers] of many different types," Mechoulam said. He was co-author on a paper by Pal Pacher reviewing the evidence that CB2 is a general protective agent. The paper listed numerous disorders in which the body's protective response involves CB2 activation. *(See illustration on next page.)* It took three slides to reproduce the list as Mechoulam spoke.

"We know that CB2 is involved in protection against inflammatory bowel disease and colitis... It protects against vascular inflammation. We see it skin disorders, bone disorders, myocardial infarctions. Decreasing inflammation in atherosclerosis. In stroke...

"I believe we should be looking very thoroughly at the mechanism of these diseases and the best way to affect those mechanisms," Mechoulam said. "There is quite a lot of work to do."

He cited three recently published papers indicating that the work is underway:

"A new cannabinoid 2 receptor agonist HU-910 attenuates oxidative stress, inflammation, and cell death associated with hepatic ischemia/reperfusion injury. Horvath *et al* 2012.

"Cannabinoid-2 receptor activation protects against infarct and oschemia-reperfusion heart injury. Wang *et al* 2012

"The action of the cannabinoid receptor type 2 reduces neutrophilic protease-mediated vulnerability in atherosclerotic plaques. Montecucco *et al* 2012."

A student of Mechoulam's developed a series of compounds that bind to the CB2 receptor (and slightly to the CB1 receptor.) They were found to improve functional recovery following brain injury. "As expected," Mechoulam said, "the CB2 receptor antagonist blocks this activity."

Unexpectedly, however, one of the new compounds, HU-914, blocks the damage but does not bind to the receptor. "The compound does not bind to the receptor and yet you can prevent its activity by the CB2 antagonist," Mechoulam repeated with emphasis.

FAAAs are signaling molecules, abundant in the brain, that include the body's own endocannabinoids, anandamide and 2-AG.

• Fatty Acid Amino Acids

FAAAs are signaling molecules, abundant in the brain, that include the body's own endocannabinoids, anandamide and 2-AG. Very few of these compounds have been studied. Several are known to have therapeutic effects. For example:

Arachidonoyl Serine —AraS (prounced Arra-ess)— lowers vasoconstriction and brain trauma effects. Arachidonoyl glycine lowers pain. Oleamide is an endogenous sleep-inducing lipid. Oleoyl serine counters osteoporosis. Palmitoyl ethnaolamide (PEA) concentrations are enhanced after damage in a specific brain region.

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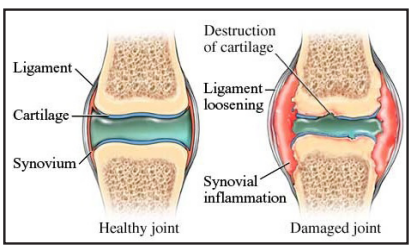
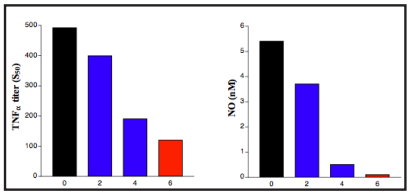
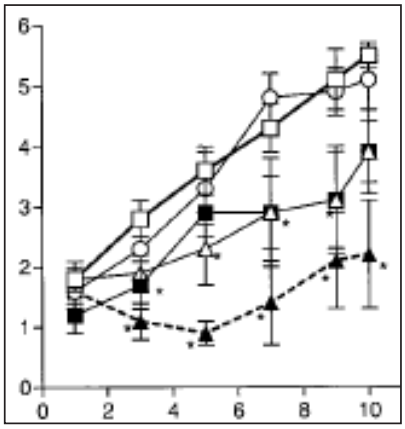


Rheumatoid Arthritis involves inflammation of the synovium —the lining of the joint space.



Effect of CBD on compounds that promote inflammation was measured by Mechoulam's colleague Ruth Gallily, working with a mouse model of Rheumatoid Arthritis. Graph at left shows decline in macrophage production of Tumor Necrosis Factor (TNF) in weeks following treatment with CBD. Graph at left shows decline in Nitrogen Oxide (NO).



"Biphasic effect" of CBD was demonstrated by experiment in which arthritic mice were given doses of zero (the controls, line marked by blank square); 2.5 milligrams per kilogram of body weight (line marked by octagon); 5mg/kg (darkened triangle); 10 mg/kg (blank triangle): and 20 mg/kg (darkened square). Vertical scale shows extent of swelling. Horizontal scale shows days after onset of arthritis. Clearly the optimal dose in this case was 5mg/kg..

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Disease	Higher eCB levels	Expression of CB2 receptors	Effects attributed to CB 2 Stimulation
Myocardial infarction ischemia/ reprefusion injury	Circulating immune cells	Myocardium	Decrease in inflammation (leukocyte infiltration) and myoccardial protection
Atherosclerosis	Serum, athero-sclerotic plaques	Infiltrating and other immune cells	Context dependent attenuation of promotion of vascular inflammation (monocyte chemotaxis, infiltration and activation) and factors of plaque stabhility; attenuation of endothelial activation and/or vascular smooth muscle proliferation.
Stroke, spinal cord injury	Serum, brain	Brain, microglia, infiltrating immune cells	Attenuation of inflammation (endothelial activation, leukocyte infiltration) and tissue injury; attenuation of motor and autonomic function deficits in a mouse model of spinal cord injury.
Heart failure, cardiomyopathy	Myocardium, cardiomyocytes	Myocardium, cardiomyocytes, endothelial cells	Attenuation of inflammation, decreased injury
Septic shock by live bacteria	Serum	ND	Decrease or increase in inflammation and itissue injhury most likely by affecting bacterial load.
Hepatic ischemia-reperfusion injury	Liver, serum, hepatocytes, Kupffer and endothelial cells	Inflammatory immune cells, activated endothelium	Attenuation of inflammation (endothelial activation, leukocyte chemotaxis, infiltration and activation), decrease in oxidative stress and tissue injury
Autoimmune hepatitis	Liver	Infiltrating immune cells	Attenuation of T lymphocyte-mediated inflammation
Nonalcoholic fatty liver disease, obesity	Liver	Hepatocytes, inflammatory cells	Enhancement of high-fat, diet-induced steatosis; inflammation or attenuation of obesity associated with age.
Liver fibrosis, cirrhosis	Liver, serum, inflammatory cells	Activated Stellate cells	Attenuation of fibrosis
Cirrhotic cardiomyopathy	Myocardium, circulating immune cells and platelets	ND	Attenuation of hypotension by decreasing liver inflammation
Liver injury and regeneration	Liver	Hepatic myofibroblasts	Reduced liver injury, accelerated regeneration
Hepatic encephalopathy	not determined (ND)	ND	Improved neurological and cognitive function in experimental models of hepatic encepholopathy
Inflammatory bowel disease, colitis, diverticulitis	Inflamed gut	Epithelial cells, infiltrating inflammatory cells, enteric nerves	Attenuation of inflammation and visceral sensitivity
Pancreatitis	Inflamed pancreas	Pancreas	Attenuation of inflammation
Nephropathy	Kidney	ND	Attenuation of inflammation (chemokine signaling and chemotaxis, inflammatory cell infiltration and endothelial activation) and oxidative/nitrosative stress
Bone disorders	ND	Osteoblasts, osteoclasts	Attenuation of bone loss by enhancement of endocortical osteoblast number and function and suppression of trabecular osteoclastogenesis
Neurodegenerative/neuroinflammatory disorders (MS, Alzheimer's, Parkinson's, Huntington's disease, spinal cord injury, neuro acquired immune deficiency syndrome)	Brain, spinal fluid	Microglia, inflammatory cells, brain lesions, neurons?	Attenuation of inflammation (microglia activation , secondary immune cell infiltration), facilitation of neurogenesis
Pain	Site of induced chronic inflammatory pain	Inflammatory cells, certain neurons	Attenuation of chronic, inflammatory pain
Psychiatric disorders (schizophrenia, anxiety and depression)	Blood, cerebrospinal fluid, brain (increased in schizophrenia decreased in brain in depression)	Glial, inflammatory cells, neurons?	Largely unexplored, in rodent models of depression/anxiety it may modulate CNS inflammation and either attenuate or promote anxiety-like behavior
Allergic dermatitis	Inflamed skin	Infiltrating immune cells	Context dependent anti-or pro-inflammatory effects in contact dermatitis.
Scleroderma/dermal fibrosis	ND	ND	Attenuation of dermal and lung fibrosis/inflammation and leukocyte infiltration
Rheumatoid arthritis	Synovial fluid, synovia	ND	Attenuation of the autoimmune inflammatory response
Allergen-induced airway inflammation (Bronchial asthma)	ND	ND	Inhibition of antigent-induced plasma extreavasation and neurogenic inflammation in airways, modulation of smooth muscle.

Proposed role of endocannabinoid-CB2 receptor signaling in select diseases incorporates results from numerous studies conducted with animals (humans, rodents, and pigs). Second column gives the part(s) of the body where elevated endocannabinoid levels were detected. Third column gives cell type(s) showing increased presence of CB2 receptors. Chart was compiled by Pal Pacher and Mechoulam in a paper published in *Progress in Lipid Research* "Is lipid signaling through receptors part of a protective system?"

In the late 1980s, after the CB1 and CB2 receptors had been discovered, Mechoulam's lab began searching for compounds in the body that would activate them. It was assumed that the receptors had not evolved to respond to plant cannabinoids. But the fact that THC and the other plant cannabinoids were lipophilic —preferring fatty molecules to water— convinced the investigators to narrow their search. The body's own cannabinoid-receptor activators would almost certainly be lipids.

"Nature is stingy," Mechoulam generalizes. "If it knows how to do something, chances are it will do it again with small changes so it will not have to learn new things."

William Devane and Lumir Hanus, fellows in Mechoulam's lab, eventually isolated arachidonic ethanolamide (AEA), one of many derivatives of arachidonic acid, and named it "anandamide," after the Sanskrit word for "supreme joy." (Devane was studying Sankrit. Mechoulam had told him "There are many synonyms for 'sorrow' in Hebrew, but considerably fewer for 'joy.'")

A second endogenous cannabinoid, 2-arachidonoyl glycerol (2-AG) was identified in 1994. Mechoulam's colleague Esther Shohami found that 2-AG levels went up nearly 10 times following closed head injury in mice. Was this because the brain had been damaged or as a protective response? A follow-up experiment showed that mice given 2-AG after closed head injury suffered about half as much damaged tissue —proving that 2-AG exerts a neuroprotective effect.

Shohami then "did one experiment too many," Mechoulam recalled ironically. "She gave arachodonoyl serine (AraS) to mice after closed head injury. She shouldn't have done that because it doesn't bind to CB1 or CB2— but, surprisingly it has the same activity as 2-AG!"

Also surprisingly, a CB2 antagonist was shown to block the effect. As would be the case with HU-914, AraS evidently works through CB2 without binding to it. AraS was recently shown to have neuroprotective effects in mice, even when administered seven days after the injury.

AraS is a fatty acid bound to an amino acid, an "F-triple-A." According to Mechoulam, "there may be hundreds of compounds formed by fatty acids binding to amino acids or their derivatives in the brain." Researchers at the University of Indiana have identified close to 100 FAAAs in recent years.

Mechoulam thinks that identifying and understanding the function of the FAAAs could shed light on the mechanisms of disease and give medical researchers extremely powerful diagnostic tools.

"Why does the body spend so much energy synthesizing so many different compounds?" Mechoulam asked. "It doesn't make sense. There should be something behind it."

He thinks that identifying and understanding the function of the FAAAs could shed light on the mechanisms of disease and give medical researchers extremely powerful diagnostic tools.

Mechoulam said, "We should be able to diagnose a disease *before* we have the physical signals, which may be too late. We should be able to analyze and find out if something is going wrong. Is it possible... If we looked at the cluster of fatty acid amino acids —the F-triple-A's— maybe we would see that there is always a change in these compounds during a pathological situation. Maybe it is not a change in one compound, say 2-AG, but there is a change in many of the compounds. Maybe we should be looking at *clusters* of compounds as biomarkers, not just one compound but 10 or 15 compounds. And modern technology allows us to do that."

A few years ago Mechoulam and Shimon Ben-Shabat reported that 2-AG binds to the receptor more readily when it is accompanied by certain fatty acid esters which, by themselves, are biologically inactive. In Freiburg Mechoulam was again urging his colleagues to think in terms of compounds acting in concert and exerting an entourage effect:

"We always look for a single biomarker, or at most, two biomarkers," he observed. Instead, he proposed, "One could look at the levels and the ratios of compounds that are present in the brain —or other organs—at the same time." Researchers could track the changing profiles of clusters in people with various disorders, in children as they grow up, in individuals during mood changes, and so forth.

"This is an observation that may have clinical importance," Mechoulam said modestly.

His hope is that changes in FAAA clusters will be detectable in blood. "Obviously one cannot look in the brain," Mechoulam said. But fortunately, researchers led by Mauro Maccarrone of the University of Teramo have confirmed "that sometimes changes in the brain are paralleled by changes in blood cells." This raises the practical possibility that the profiles of various FAAA clusters can be tracked and correlated with pathology, aging, mood change, etc.

"It is tempting," Mechoulam wrote in the abstract of his ICRS presentation, "to assume that the huge possible variability of the levels and *ratios* of substances in such a cluster of compounds may allow an infinite number of individual differences —the raw substance which of course is sculpted by experience."

He said he had asked a statistician if 200 compounds could yield eight billion distinct personalities. "The answer was 'Of course.'"

At age 82, Dr. Raphael Mechoulam is looking ahead with undiminished clarity and creativity. "These are our plans for the next 50 years," he told his assembled colleagues. "I hope that many of you will join me in looking at these compounds."

Notes From the Meeting

Here we have the man who coined the term *endocannabinoid* reminding his colleagues not to be constrained by that term but to study the whole assemblage of FAAAs and fatty acid ethanolamides and glycerol esters —the extended endocannabinoid family, with its precursors and derivatives.

"Fatty acids are dabs of molecular goo," explains Dr. J. "They intermesh with each other to form membranes, so you tend to think of them as building blocks, not as transmitters. But those membranes give us the surfaces to receive drugs. The external coating of every cell in our bodies, including all the neurons, is a fatty acid bi-layer. What allows the transmission of the outside world to the inside world is a gatekeeper of fatty acids that have receptors implanted in them.

"Raphe encapsulated the wonderment of all the science we don't know," said Dr. J (Many of the ICRS scientists freely refer to the great man as "Raffee," which is short for Raphael. He's their friend, accessible, and does not engage in ranking.) "One of the miraculous things about the cannabinoids is that they're only found in the *Cannabis* plant.

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But the precursors are found in other plants. Olivetol is in lichens! What is the lichen using olivetol for? It might be worth looking into," Dr. J added, sounding a bit like Mechoulam himself.

We asked this attentive scientist what he considered most newsworthy about the 2012 ICRS meeting. After reflecting a few seconds, he said, "The confirmation that cannabinoids are anti-neoplastic compounds. People used to say the cannabinoids had 'anti-cancer potential.' Now they're talking about demonstrated 'anti-cancer properties.' Not just that they relieve pain and nausea. Compounds extracted from natural cannabis can stop cancer in its tracks.

"Also newsworthy, in an invisible way, is that you don't hear reports of toxicity. You don't hear any cannabinoid researchers saying, 'We gave them this and the animals died..."

Many of the presentations in Freiburg involved the areas of research Mechoulam discussed in his plenary address, as if the authors had gotten a head start on the next 50 years. Dutch researchers from Wageningen University presented evidence that "fatty acid metabolites from the dietary fish oil DHA contribute to the beneficial effects of long chain omega-3 polyunsaturated fatty acids."

Primary investigator Jocelijn Meijerink also reported that DHEA (a DHA-related conjugate) binds directly to the CB-2 receptor and functions as a "fish-oil-derived modulator of inflammation." In other words, eating fish or fish oil supplements can enhance endocannabinoid tone and improve one's overall health.

Israeli scientist Sharon Anavi-Goffer reported that administration of a CB-1 antagonist induced ADHD-like behavir in adult mice. She also found that pure THC alleviated ADHD symptoms in schoolchildren.

Studies indicated that a small amount of THC can have a potent therapeutic impact. Steve Kinsey from West Virginia University found that THC "protects against gastric inflammatory tissue damage" in animal experiments "at doses that are insufficient to cause cannabimimetic behavior effects," i.e., that are not psychoactive.

Yosef Sarne from Tel Aviv University reported that extremely low doses of THC protect against "cognitive deficits and induce long-lasting biochemical changes in the mouse brain.

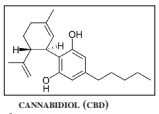
Tamas Biro, a Hungarian scientist at the University of Debrecen, reported that the inhibition of fatty acid amide hydrolase (FAAH), the enzyme that breaks down the body's own cannabinoids, exerts "complex 'anti-acne' effects" on human cell lines.

According to Mauro Maccarone of the University of Teramo in Italy, estrogen activates expression of the gene that encodes FAAH —the implication being that estrogen imbalance (precipitated by environmental toxins and other poisons) will skew cannabinoid-receptor signaling.

A collaborative effort involving researchers at MIT and Kings College in London concluded that compounds which activate cannabinoid receptor signaling (agonists) "markedly increase neural progenitor migration," while cannabinoid receptor antagonists significantly impair stem cell production and migration in the postnatal mammalian brain.

CBD Studies

Italian investigators led by Luciano De Petrocellis described cannabidiol as "the most efficacious inducer of apoptosis" in prostate cancer.

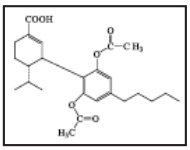


CANNABIDIOL (CBD)

Ditto against colon cancer, according to Tara Macpherson Dr. Karen Wright from Lancaster University and John Westwick from Novartis/UK. They found that CBD works by "limiting the cells' respiratory capacity and ability to generate ATP, which leads to cell death rather than cell cycle inhibition." The CB1 receptor is not involved in the process, which occurs "under mitochondrial stress conditions" and only when a certain narrow dose is delivered.

The effect of CBD on mitochondrial functions was studied by Israeli resaearchers led by Neta Rimmerman of the Weizmann Institute of Science. They found that "CBD treatment led to significant changes in mitochondrial morphology (mainly swelling)." CBD interacts with the outer-mitochndrial mebrane protein. It increases formation of reactive oxygen species (ROS) in a dose dependent manner.

A team led by Christeene Haj in Ruth Galily's lab at Hebrew University of Jerusalem developed an analog of CBD that is an effective anti-arthritic agent in the mouse model of the disease (induced by injection of collagen). The analog, patented as HU (for Hebrew University) 444, reduced swelling more potently than unadorned CBD.



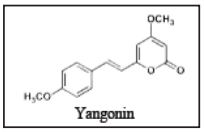
HU-444

Compound in Kava Affects CB1 Receptor

The Kava plant has long been used as a relaxing, social beverage by South Pacific Islanders who make a kava drink by grinding the roots. Now research initiated by Istvan Ujvary of iKem BT, Budapest, has identified an active ingredient in kava —yangonin— that has a strong affinity for the CB_1 receptor.

In a study described by Ujvary at the 2012 ICRS meeting, five natural kavalactones (compounds unique to the Kava plant) and nine synthetic analogues were tested for affinity to the CB_1 and CB_2 receptors, and their ability to inhibit FAAH and MAGL (the enzymes that break down anandamide and 2-AG).

Only yangonin showed a marked affinity for a cannabinoid receptor (CB_1). None of the compounds tested inhibited FAAH or MAGL hydrolytic activity. The authors concluded, "The observed affinity of yangonin to the CB1 receptor, though moderate, suggests that the endocannabinoid system might be involved in the complex human psychopharmacology of the traditional kava drink and the anxiolytic preparations obtained from the kava plant."



Yangonin

Yangonin could turn out be an agonist, an antagonist, or an inverse agonist at the CB1 recptor. More studies are warranted, said Ujvary.

Daniel Nomura photo: Paul Kirchner Studios

Wonders of MAGL Inhibition

The enzyme that breaks down the endocannabinoid 2-AG is monoglyerol lipase (abbreviated MAGL, pronounced "Mag-Ell"). It was identified in Benjamin Cravatt's lab at Scripps Research Institute in La Jolla by Daniel Nomura, who had been systematically studying enzymes inhibited by certain pesticides. (Agricultural insecticides don't just eliminate the enzyme that's essential for the pest's reproduction; they prevent the synthesis of other enzymes, too. One animal's collateral damage is another's research opportunity.)

Cravatt and Nomura subsequently showed that inhibiting MAGL production leads to enhanced cannabinoid-receptor activation and reduced cancer cell growth.

The paper by Nomura, Tarak Samad, and Cravatt at this year's ICRS meeting, "Endocannabinoid Hydrolysis Generates Brain Prostaglandins That Promote Neuroinflammation" describes MAGL as a link between the endocannabinoid and prostaglandin signaling networks.

In the brain and central nervous system, the breakdown of 2-AG by MAGL produces arachidonic acid, which in turn produces prostaglandins that promote inflammation. But prostaglandins are not produced when MAGL breaks down 2-AG in the gut (suggesting that a MAGL-inhibiting painkiller would not be hard on the stomach). The authors foresee "a potentially safer way to suppress the proinflammatory cascades that underlie neurodegenerative diseases" such as Parkinson's, Alzheimer's and multiple sclerosis.

What Role Does GPR55 Play?

Cannabinoid researchers have been studying the pharmacology and the role played in the body of GPR55, a G-protein-coupled receptor implicated in tumor formation and angiogenesis, cancer cell proliferation, and pain transmission. At the 2012 ICRS meeting June Penman and coworkers from the University of Dundee in Scotland presented data suggesting that "activation of GPR55 can influence the actin cytoskeleton" in certain cancer cell lines. They raised "the possibility that the endogenous ligand LPI may be an intrinsic regulator of cancer metastasis."

Metabolically, LPI is closely related to 2-arachidonoylglycerol (2-AG), the endocannabinoid that binds to the CB1 and CB2 receptors. Phosphatidylinositol, a natural lipid, is a direct precursor to both LPI and dicylglycerol (DAG); the latter is converted enzymatically (by DAGLipase) into 2-AG. These intimate metabolic relationships led scientists from University College in London to suggest in 2006 that GPR55 should be considered a third cannabinoid receptor.

But there are significant structural differences between GPR55 and the two known cannabinoid receptors. GPR55 is genetically distinct from the cannabinoid receptors, sharing only about 14% of their amino acid sequences. Moreover, many of GPR55's binding sites are hydrophilic, meaning they bind to water soluble ligands, whereas the CB1 and CB2 cannabinoid receptors are hydrophobic and bind only with lipids.

GPR55 was first identified and cloned in 1999 by scientists at the University of Toronto. Eight years later, scientists from Teikyo University determined that lysophosphatidylinositol (LPI) is the principal endogenous ligand that binds to and activates GPR55.

LPI is produced by various types of cells. A 2004 study by researchers at the University of South Florida determined that high concentrations of LPI in the body (as well as partial agonists of GPR55 such as lysophosphatic acid and sphingosine-1-phosphate) are markers of ovarian cancer. High concentrations of LPI increase GPR55 receptor transmission. And heightened GPR55 receptor transmission has been linked to many types of cancer.

Experiments have sought to determine which cannabinoid compounds affect GPR55 and how.

Various experiments have sought to determine which cannabinoid compounds affect GPR55 and how. A January 2012 study by scientists at the University of Aberdeen in Scotland found that several plant cannabinoids, including THC, CBD and CBG, and their respective acid and varin compounds, bind to the GPR55 receptor. The varin compounds –Δ9-tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), and cannabigerovarin (CBGV)– were shown to be particularly potent inhibitors of GPR55 signaling. [A "varin" is created by a three-carbon side-chain attaching to a given cannabinoid.]

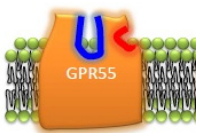
The Aberdeen group determined that the synthetic CB1 antagonist drug Rimonabant increases GPR55 receptor signaling. Similarly, the CB1 antagonist/inverse agonist AM251 is an agonist at GPR55.

The Aberdeen group explored an important aspect of receptor physiology —the key differences between orthosteric and allosteric binding sites. These differences may help to explain why studies of cannabinoid-GPR55 interactions have produced conflicting data.

An orthosteric binding site is the standard receptor binding spot: the main endogenous ligand of a receptor –such as LPI to GPR55– activates the receptor by binding orthosterically, or directly, like lock and key.

Allosteric interactions, on the other hand, can alter how the orthosteric site functions. Allosteric binding can change the shape of a receptor and influence how it signals. It may be that CBD and other phytocannabinoids, especially the varins, weaken or block GPR55 through an allosteric mechanism, which inhibits the receptor's orthosteric binding affinity with its principal ligand, LPI.

At the same time, however, a cannabinoid compound that blocks LPI from binding with GPR55 by altering the shape of the receptor might also change the shape of the receptor in such a way as to enhance GPR55's binding affinity with other endogenous agonists or partial agonists.



GPR55 receptor has an orthosteric binding side (top center) and an allosteric site (top right).

The protean aspect of allosteric binding could be a factor in the broad range of effects triggered by cannabinoid-GPR55 interactions. The way plant cannabinoids impact GPR55 could shift, depending on the presence of different biochemical cues.

—Martin A. Lee and Adrian Devitt-Lee