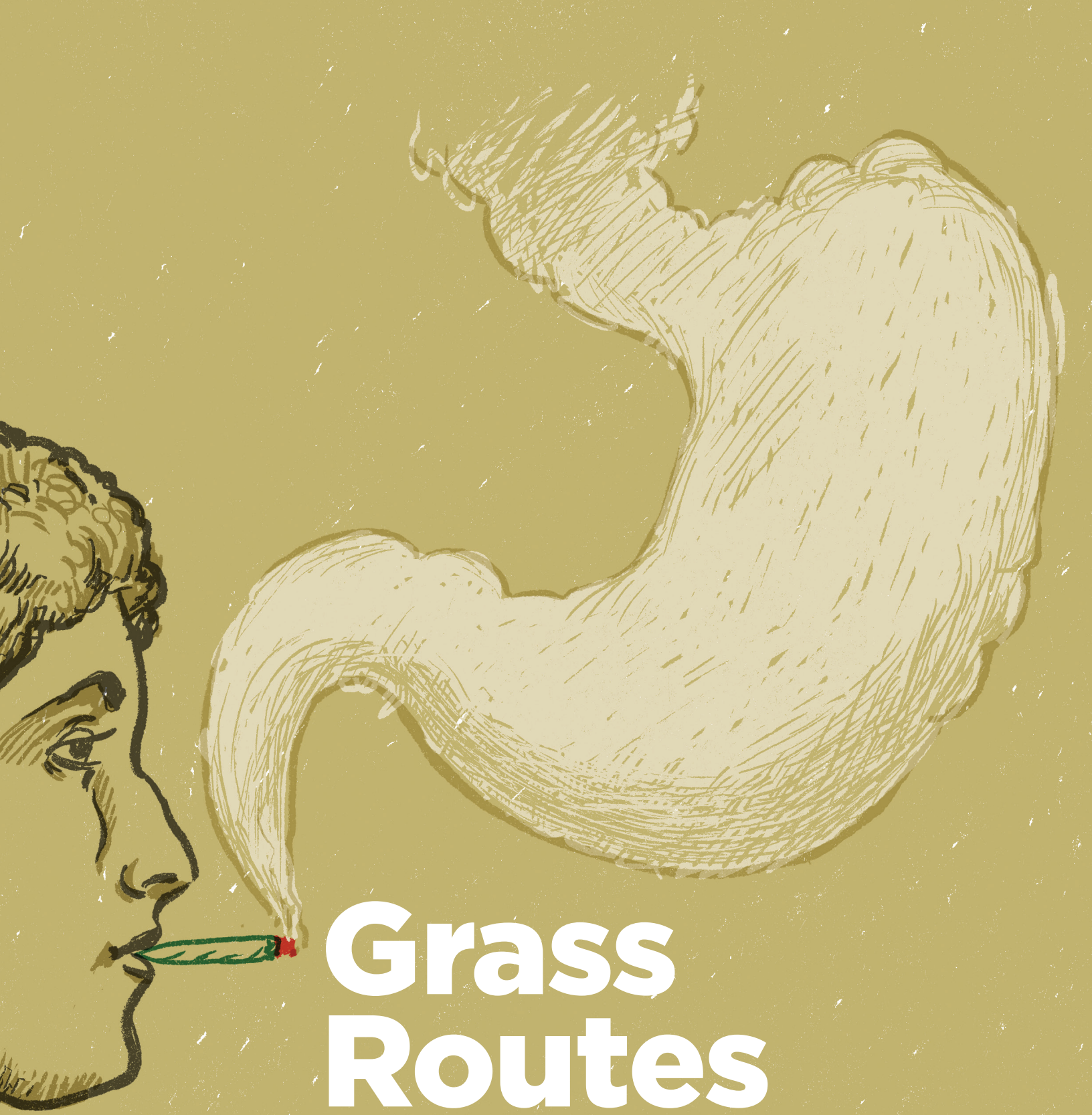


Grass Routes

Researchers are discovering a suite of new locations and functions of endocannabinoid receptors that play roles in sickness and in health.

BY MEGAN SCUDELLARI

Neuropharmacology postdoc Nick DiPatrizio was stumped. His advisor, University of California, Irvine, researcher Daniele Piomelli, had discovered eight years earlier that hungry rats have high levels of endocannabinoids, endogenous molecules that bind to the same receptors as the active ingredient in marijuana.

Now, in 2009, DiPatrizio was trying to identify exactly where and how those molecules were controlling food intake in rats. But under specific feeding conditions, he couldn't locate any changes in endocannabinoid levels in the brain, which is flush with endocannabinoid receptors and the obvious place to look for behavioral signals.

It has been known for some time that the brain can modulate the gut. With endocannabinoids, it appears the gut can also modify the brain.

Piomelli gently chastised his mentee. "He said, 'You're being neurocentric. Remember, there's a body attached to the head. Look in the other organs of the body,'" recalls DiPatrizio. So the young scientist persisted, and eventually discovered that hunger—and the taste of fat—leads to increased endocannabinoid levels in the jejunum, a part of the small intestine. Endocannabinoid signaling in the gut, not the brain, was controlling food intake in the rodents in response to tasting fats.[1]

The evolution of endocannabinoid research has mirrored DiPatrizio's early thinking: ever since the first endocannabinoid receptor was identified in the late 1980s, the field has been overwhelmingly focused on the central nervous system. The main endocannabinoid receptor, CB_1, was first discovered in a rat brain and is now known to be among the most abundant G protein–coupled receptors in neurons there. Plus, cannabis is well-known for its psychotropic effects. "That has led the research field to be very CNS-oriented," says Saoirse O'Sullivan, who studies endocannabinoids at the University of Nottingham in the U.K.

But recent work has provided evidence that the endocannabinoid system—a family of endogenous ligands, receptors, and enzymes—isn't exclusive to the brain. It is present everywhere in the body that scientists have looked: the heart, liver, pancreas, skin, reproductive tract, you name it. And disrupted endocannabinoid signaling has been associated with many disorders, including diabetes, hypertension, infertility, liver disease, and more. "There is so much that's still unknown about this system. It looks to be regulating every physiological system in the body," says DiPatrizio.

Now an assistant professor at University of California, Riverside, School of Medicine, DiPatrizio has trained his whole research program on the gut, where the endocannabinoid system appears to be a major player in human health and disease. In January, his lab suggested that endocannabinoid signaling in the gut drives the overeating characteristic of Western diets. In a rodent model, chronic consumption of a high-fat and high-sugar diet led to elevated levels of endocannabinoids in the gut and blood, promoting further consumption of fatty foods. Blocking endocannabinoids from their receptors decreased overeating in the animals, his team found.[2]

Because of that link to appetite, pharmaceutical companies have sought to target the endocannabinoid system to create the ultimate diet pill, a drug to reduce appetite or treat metabolic disorders. Those efforts have recently been subdued by two tragic and highly visible failures. (See "On Trial, Off Target" on page 38.) But some scientists still hope that by understanding the true nature of this system, they might identify new treatments, especially for conditions related to gut health and metabolism.

"We are now at a point where you have to understand how [endocannabinoids] can be so relevant in so many areas—literally everywhere in the body," says Mauro Maccarrone, head of biochemistry and molecular biology at Campus Bio-Medico University of Rome, Italy, who has studied the molecules since 1995. "There must be a reason why these endocannabinoids are always there."

In the weeds

Researchers describe the endocannabinoid system as the most complicated and most ubiquitous signaling system in our bodies, yet no one knew it was part of human physiology until the 1980s. And that realization came from an unusual source—an oft-derided effort to understand how marijuana gets us high.[3]

In 1964, researchers seeking to understand the psychoactive component of the cannabis plant identified the compound Δ^9-*tetrahydrocannabinol,* or THC.[4]

More than two decades later, in 1988, investigators found the first direct evidence of an endogenous signaling system for THC—a receptor in the rat brain that bound a synthetic version of THC with high affinity.[5] Blocking the receptor with a chemical antagonist in humans effectively blocks the high typically experienced after smoking marijuana.

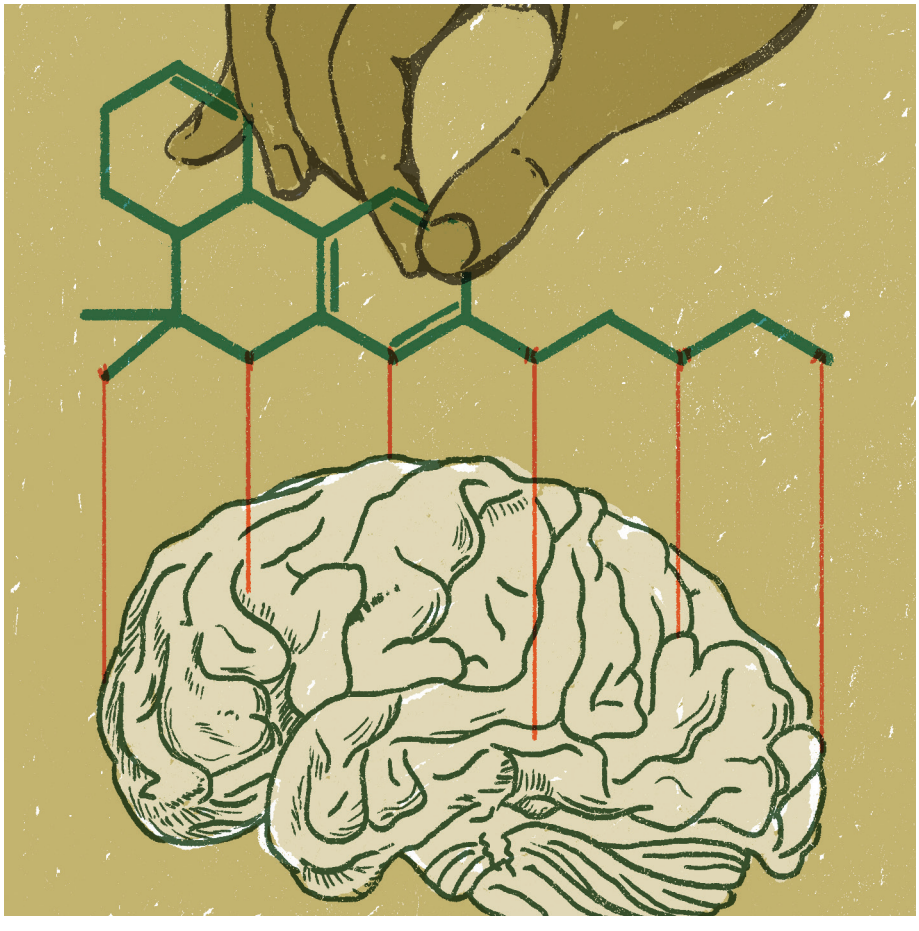
The receptor, called CB_1, was subsequently identified in other mammalian brains, including those of humans, and appeared to be present in similar density to receptors for other neurotransmitters, including glutamate, GABA, and dopamine.[6] A second cannabinoid receptor, CB_2, was discovered in 1993.[7] This receptor was first isolated in the rat spleen. That surprising finding was an omen of things to come; the endocannabinoid system functions far afield from the brain, practically everywhere in the body.

The presence of these receptors sparked a quest to find natural ligands that bind to them. The first endocannabinoid identified, a fatty acid-based agonist for both receptors, was named anandamide, based on the Sanskrit word *ananda* meaning "inner bliss." A second agonist, 2-arachidonoylglycerol (2-AG), did not get so groovy a name, but did appear to be present at high levels in normal mammalian brains.

By 1995, the so-called "grass route" was complete: over three decades, researchers had identified THC, its endogenous receptors, and endogenous ligands for those receptors. Maccarrone suspects that endocannabinoids are among the oldest signaling molecules to be used by eukaryotic cells. His team recently showed that anandamide and its related enzymes are present in truffles, delectable fungi that first arrived on the evolutionary scene about 156 million years ago, suggesting endocannabinoids evolved even earlier than cannabis plants.[8]

"They are kind of a master signaling system, and other signals have learned to talk to these lipids," says Maccarrone. In the brain, endocannabinoids interact with other neurotransmitters; in the reproductive tract, with steroid hormones; in the muscles, with myokines; and so on.

But even though researchers have documented the existence of the endocannabinoid system throughout the body, they still don't really know what role it plays outside the brain, where it is involved in synaptic signaling and plasticity. In healthy, non-obese animals, there is typically no consequence to knocking out endocannabinoid receptors in peripheral organs. "There is



> We are now at a point where you have to understand how endocannabinoids can be so relevant in so many areas—literally everywhere in the body.
>
> —Mauro Maccarrone
> Campus Bio-Medico University of Rome

no detectable effect on any important biological function," says George Kunos, scientific director of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) at the National Institutes of Health.

What's the buzz?

The one exception to this functional black box is the gastrointestinal tract. The idea that cannabis—or, by extension, endogenous cannabinoids—affects the gut is not surprising. Preparations derived from marijuana plants have long been used to treat digestive conditions such as inflammatory bowel disease and vomiting. Even before CB_1 was discovered, scientists had suggested that cannabinoids regulate the motility of the gastrointestinal tract—the orchestrated movements of muscles that churn and move food through the intestines. For example, in 1973, Australian researchers showed that oral ingestion of THC slowed the passage of a meal through the intestines of mice.[9] Conversely, knocking out parts of the system is associated with increased movement of food through the colon, a common symptom of irritable bowel syndrome (IBS). These pathways are conserved among many species.[10]

Both CB_1 and CB_2 receptors are present and active in the gut, though they appear to be involved in different gut functions. At the University of Calgary, Keith Sharkey and colleagues found that increased intestinal motility in the inflamed gut was reversed when CB_2 receptors, but not CB_1 receptors, were activated.[11]

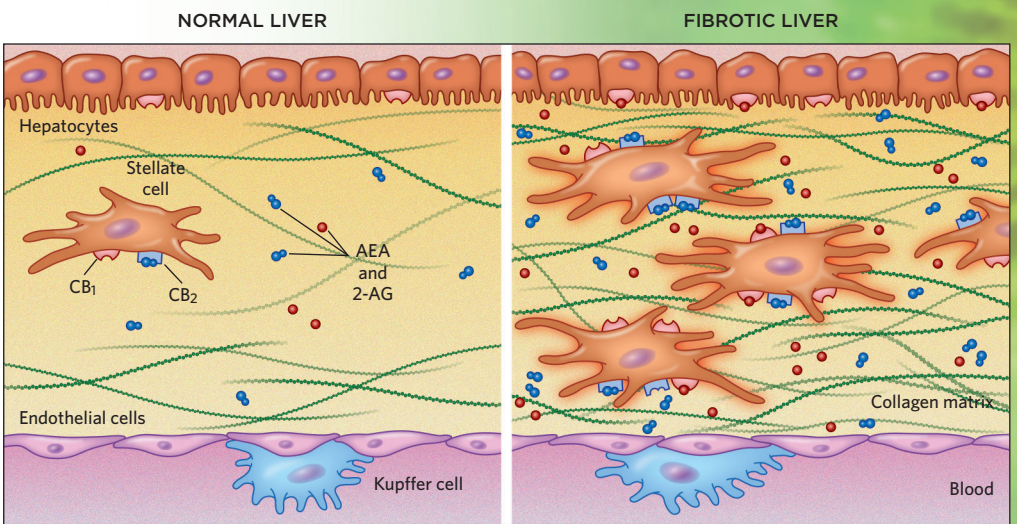
To make things even more complicated, there is a group of nonclassical receptors that interact with endocannabinoids in the gut, says Jakub Fichna, head of the department of biochemistry at the Medical University of Lodz in Poland. His lab studies the role of these receptors in inflammatory bowel disease (IBD) and IBS. Depending on the conditions in the gut, some of these nonclassical receptors don't even need an agonist or antagonist to become active, Fichna says. "It can even be the change in pressure or pH of the neighborhood. For example, if you have inflammation, most of the time you have decreasing pH, and this is already enough for some of the endocannabinoid receptors to be activated."

Endocannabinoids and their receptors also appear to be involved in gastric secretions, ion transport, and cell proliferation in the gut. And then there is appetite. Marijuana users often experience the "munchies"—a sharp and sudden increase in appetite after inhaling or ingesting the drug. Kunos wondered whether endocannabinoids cause a similar increase in appetite. In 2001, with the help of collaborators, he confirmed the suspicion: endocannabinoids acting on CB_1 receptors promoted appetite, and mice with CB_1 receptors knocked out ate less than their wild-type littermates.[12]

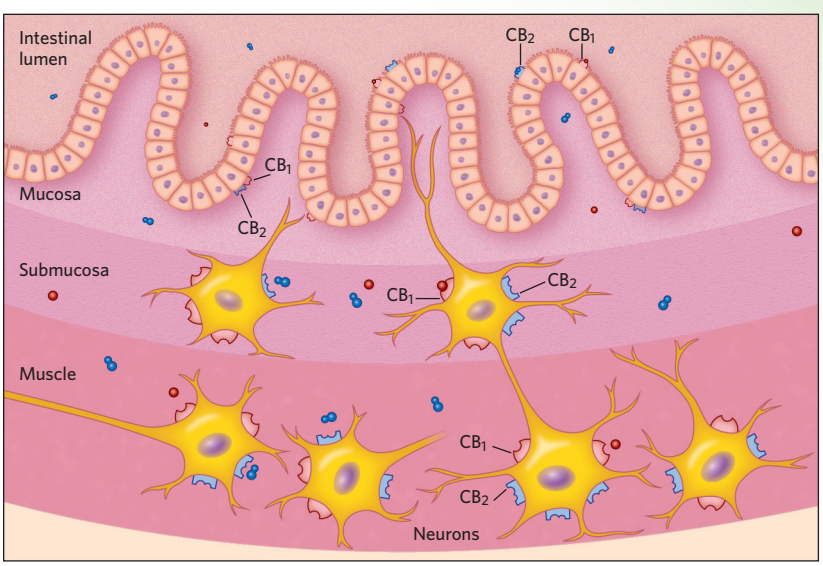
Additional research has supported that idea that endocannabinoids act as a general appetite-promoting signal. And as DiPatrizio's work showed, endocannabinoids control food intake not exclusively via the brain, but by way of signals gen-

ENDOCANNABINOID SIGNALING IN THE BODY

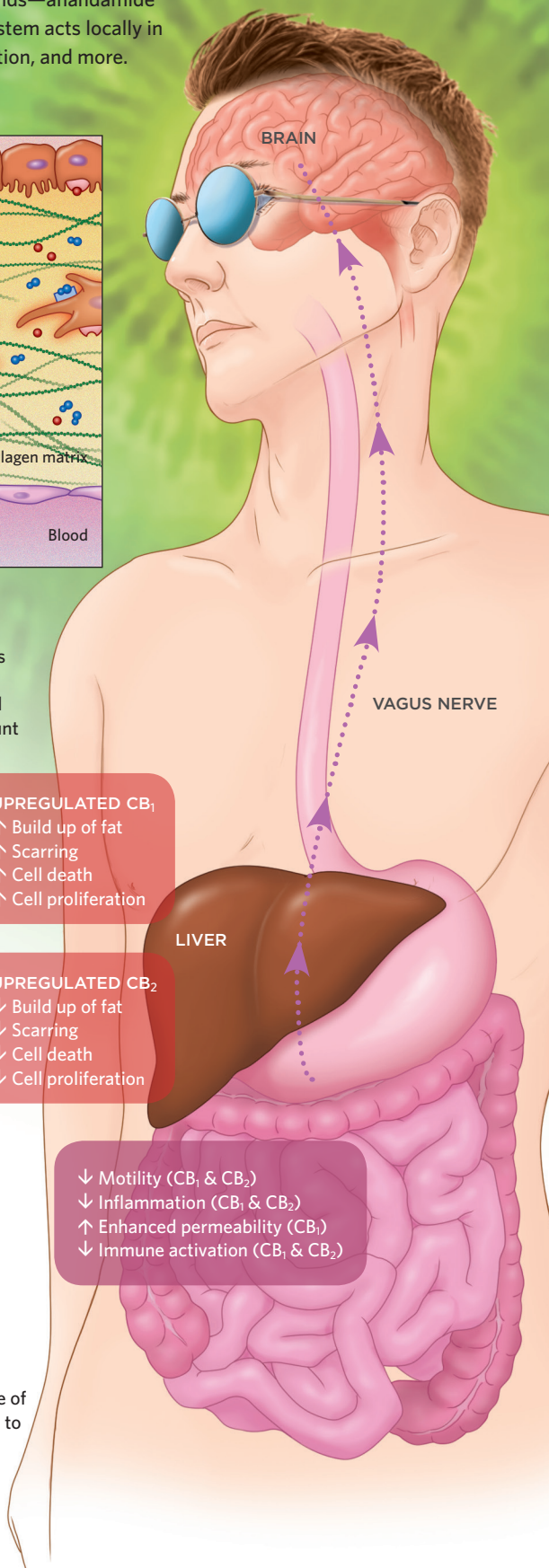
The two classical cannabinoid receptors, CB_1 and CB_2, are expressed by enteric neurons, immune cells, and other cell types within the gastrointestinal tract. The gut and the liver also synthesize two key ligands—anandamide (AEA) and 2-arachidonoylglycerol (2-AG)—for those receptors. Combined, this signaling system acts locally in the gut and liver, but also communicates with the brain to affect food intake, pain, inflammation, and more.



In the liver, endocannabinoids are thought to act almost like hormones, stimulating cell division at some times, cell death at others. In the healthy liver, expression of CB receptors is very low, but in a diseased liver expression increases, and endocannabinoid ligands are released from all four cell types shown here. Many ligands are produced and bind to CB_1 receptors, causing lipid accumulation and insulin resistance in hepatocytes, and increased proliferation of activated stellate cells, the major cell type involved in fibrosis (scarring) of the liver. Blocking CB_1 receptors with drugs decreased the amount of fibrosis in mouse models.



Both CB_1 and CB_2 regulate the rhythmic contractions of the intestinal tract, called gut motility. In the healthy gut, CB_1 predominates, but during intestinal inflammation, CB_2 also contributes to motility. Conditions such as inflammatory bowel disease and celiac disease often exhibit increased prevalence of these receptors, which results in decreased motility. Endocannabinoid signaling has also been shown to reduce inflammation, increase the permeability of gut epithelial cells, and signal hunger to the brain.



erated in the gut. It's a simple hypothesis with big implications for the management of obesity and other metabolic syndromes.

During his postdoc, DiPatrizio found that when rodents tasted dietary fats (just tasted, not swallowed), endocannabinoid levels increased in the rat small intestine—and nowhere else in the body. A CB_1 receptor antagonist blocked that signal, leading the rodents to decrease their ingestion of fatty foods. "This suggests to us that this is a very important and critical mechanism that drives food intake," says DiPatrizio.

From an evolutionary perspective, having a positive feedback mechanism for fat intake makes sense, he adds. When an animal in the wild detects high-energy foods, it is beneficial to stock up. However, that's not true for people in today's developed countries. "There's no period of famine. It's feast all the time, so now the system can drive us to overconsume," says DiPatrizio.

Sharkey sees the system as a regulator of homeostasis within the body, especially considering its roles in maintenance of food intake, body weight, and inflammation. "It seems to be very important in the conservation of energy," says Sharkey. "But in modern Western society in particular, those are the things that appear to have been dysregulated."

Times of trouble

Although the job of the endocannabinoid system remains mysterious in healthy tissues outside the brain and gut, diseases reveal clues. In obesity, both CB_1 and CB_2 receptors are upregulated throughout the body, including in the liver and in adipose tissue. And the activation of CB_1 receptors increases food intake and affects energy metabolism in peripheral tissues. In type 2 diabetes, endocannabinoids and their receptors are upregulated in circulating macrophages and contribute to the loss of pancreatic beta cells, which store and release insulin.

Interestingly, chronic marijuana users have no documented increased incidence of diabetes or obesity. Researchers speculate this is because chronic use results in downregulation of CB_1 receptors—a form of pharmacological tolerance. Another

ON TRIAL, OFF TARGET

The endocannabinoid system has proven a tantalizing, if elusive, target for the pharmaceutical industry, especially for conditions related to appetite and gut health. Sanofi-Aventis was the first to market an antiobesity drug targeting endocannabinoid receptors. In 2006, the European Commission approved the CB_1 antagonist rimonabant (Acomplia) as a treatment to curb hunger. It did so effectively, but as a wider population of people began using it, dangerous side effects emerged. A small percentage of users suffered from serious psychiatric symptoms, including suicidal thoughts.[17] In 2008, the European Medicines Agency recommended suspension of the drug, and the company withdrew it from the market.

That withdrawal halted the development of the whole class of CB_1 antagonists, says George Kunos, scientific director of the National Institute on Alcohol Abuse and Alcoholism. Yet the side effects should have been predictable, he argues, as CB_1 receptors play an important role in brain reward pathways. Blocking them, he says, therefore is likely to cause an inability to feel pleasure.

Last January, the field was dealt a second blow. In France, six participants in a Phase 1 study of a compound known as BIA 10-2474 were hospitalized with neurological symptoms. Portuguese pharmaceutical company Bial was developing the drug as a candidate to treat a number of neurological disorders, including anxiety. But within days of receiving multiple daily doses of the drug, one participant was declared brain-dead, while others developed severe lesions on their brains.

BIA 10-2474 is an inhibitor of fatty acid amide hydrolase (FAAH), a key enzyme that breaks down endocannabinoids. Researchers had hoped that by targeting a downstream part of the endocannabinoid system, rather than the receptors themselves, they might avoid off-target effects in the brain and elsewhere. That was not the case. "That, again, scared regulators and the industry away from consideration of that system," says the University of Calgary's Keith Sharkey, who was not involved in the trial. There is still potential for drug development in the field, he emphasizes, but only under carefully controlled conditions with drugs that can be restricted to specific sites of action.

possibility, explored by Sharkey and colleagues in 2015, is that chronic THC exposure alters the gut microbiome, affecting food intake and preventing weight increase.[13] In liver disease, upregulation of CB_1 appears to contribute to cell death and the accumulation of scar tissue (fibrosis).[14] (See "Endocannabinoid Signaling in the Body" on page 37.)

Yet there remains debate as to whether endocannabinoid receptors are always the bad guys in disease. In some cases, endocannabinoid signaling even appears to be therapeutic. Animal studies suggest endocannabinoids are effective pain relievers, and the system has anti-inflammatory properties in certain contexts. In IBD, Sharkey's team found that activation of both CB_1 and CB_2 receptors resulted in reduced inflammation, suggesting the system may be activated as a protective force. Likewise, CB_2 activation appears to be anti-inflammatory in cases of atherosclerosis, says O'Sullivan, who focuses on endocannabinoids in the cardiovascular system. "It's a bit of a rescue receptor," she says. "In times of trouble, it gets upregulated." And several tantalizing studies suggest cannabinoids—from plants or from synthetic compounds that mimic botanical molecules and the body's own—might directly inhibit cancer growth by inducing cell death in tumor cells.

But the very thing that makes the endocannabinoid system so interesting—its ubiquity and varied roles in the body—is also what makes it a difficult drug target. Within the last 10 years, two drugs targeting the endocannabinoid system proved to have dire side effects in humans when the compounds crossed the blood-brain barrier. Off-target effects in other organ systems could also have long-term consequences, such as damage to a young woman's reproductive system. Indeed, in a recent review of the pharmacology of 18 different CB_2 ligands as potential drug candidates, Maccarrone and a large team of European researchers, in collaboration with Roche, concluded that just three of the compounds (none of which were developed by Roche) merited additional preclinical or clinical studies.[15] Many of the other compounds engendered too many off-target effects.

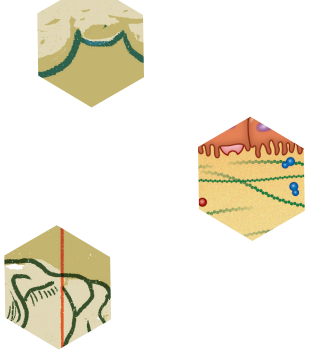
Researchers are now working toward second-generation drugs that more specifically target peripheral systems. "If the scientific community faces the challenge of really understanding how to direct certain drugs to the right target, then we could have wonderful drugs for the future," says Maccarrone. Most of those compounds are in preclinical trials, though Kunos hopes to have an Investigational New Drug approval from the US Food and Drug Administration soon for one agent his team has been working on as a possible treatment for nonalcoholic fatty liver disease. The compound does not penetrate the brain and is designed to accumulate in the liver, which may explain its efficacy in treating liver disease without causing psychiatric side effects in animal models, says Kunos.[16]

If researchers can figure out how to avoid the devastating off-target effects, there is one more reason why endocannabinoids may effectively help treat disease: they provide an indirect link to the brain. "We've known, for some time, that the brain can modulate the gut," says Sharkey. With endocannabinoids, it appears the gut can also modify the brain. It is now clear, for example, that there are very active communication pathways originating from peripheral nerves in the gut that are able to modulate brain function. Numerous studies suggest the vagus nerve is a major information highway between the gut and brain.

DiPatrizio is studying those communication pathways and hopes to identify ways to regulate feeding without ever getting near the brain with a drug. The research complements other evidence showing that the gut is able to modulate proinflammatory cytokines in the blood and even influence central nervous system disorders.

"We believe we can remotely control the brain from the gut, safely," says DiPatrizio. "That's why, once again, [endocannabinoid receptors] are very attractive targets." ■

Megan Scudellari is a science journalist based in Boston, Massachusetts.



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