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New study sheds light on painkilling system in brain

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Repeatedly boosting brain levels of one natural painkiller soon shuts down the brain cell receptors that respond to it, so that the painkilling effect is lost, according to a surprising new study led by Scripps Research Institute and Virginia Commonwealth University scientists. The study has important implications for drug development.

The natural painkiller, 2-AG, is one of the two major "endocannabinoid" neurotransmitters. The other, anandamide, can be kept at high levels in the brain without losing its therapeutic effects, and researchers had hoped that the same would be true for 2-AG.

"One implication is that maximally elevating 2-AG levels in the brain might not provide a straightforward path to new pain drugs," says Benjamin F. Cravatt III, PhD, professor and chair of the Department of Chemical Physiology and member of the Skaggs Institute for Chemical Biology at Scripps Research in La Jolla, California, who led the study with Aron Lichtman, PhD, a professor of pharmacology and toxicology at Virginia Commonwealth University in Richmond, Virginia. "But we remain optimistic that more modest elevations in 2-AG could produce sustained pain relief. Perhaps more importantly, on a basic science level, we've been able to tease apart a key difference between the two major endocannabinoid signaling pathways, since one can maximally elevate anandamide without observing tolerance."

The report appears in the August 22, 2010 issue of Nature Neuroscience.

A Better Chill Pill

Like the opioid system, the endocannabinoid system was discovered as a result of humans identifying a plant - in this case marijuana (cannabis sativa) - that artificially boosts its activity. Marijuana's main active ingredient, THC, typically reduces pain and anxiety. Researchers have sought to develop drugs that reproduce such therapeutic effects while leaving out THC's unwanted side effects - which include memory impairment, locomotor dysfunction, and possibly addiction.

Cannabinoid research received a boost in 1990 with the description of the main cannabinoid receptor in the brain, CB1, and a few years later with the discoveries of the body's own (endo-) cannabinoids, anandamide and 2-AG, which exert most of their effects by binding to CB1. Cannabinoid receptors are now known to be widely distributed in the brain, and when activated by anandamide or 2-AG, tend to calm the activity of the neurons where they reside. However, researchers so far have been unable to develop artificial cannabinoids that bind to CB1 without producing unwelcome THC-like side effects.

An alternative strategy has been to boost levels of the body's own cannabinoids by inhibiting the enzymes that normally break them down. And so far this has worked for anandamide. Inhibitors of its breakdown enzyme, fatty acid amide hydrolase (FAAH), have been shown to boost anandamide levels and reduce pain and inflammation without adverse side effects in animal tests and early clinical trials.

A similar strategy for boosting 2-AG may be promising, too, especially since 2-AG levels in the brain are naturally higher than anandamide's. Two years ago, the Cravatt and Lichtman laboratories jointly reported the development of an inhibitor of 2-AG's breakdown enzyme, monoacylglycerol lipase (MAGL). When administered to mice, it boosted their brain levels of 2-AG on average by a factor of eight, and

produced a pain-killing effect comparable to that of FAAH inhibitors.

Diminishing Returns

Now the two labs report that 2-AG's pain-killing effect disappears after six days of treatment. "When you continually stimulate the endocannabinoid system by maximally raising 2-AG levels, you effectively desensitize the system," says Cravatt.

In one experiment, an injection of the MAGL inhibitor into mice showed evidence of pain relief on standard tests, but after six consecutive daily injections the drug could no longer achieve this effect. These chronically treated mice also lost much of their sensitivity to THC and to a synthetic CB1-binding compound, and showed a classic sign of drug dependency—when abruptly withdrawn from 2-AG's influence by having their CB1 receptors blocked, they developed paw flutters - a murine version of the shakes.

"When we investigated at the molecular level, we found that the number of CB1 receptors in the mouse brains had been reduced," says Jacqueline Blankman, a graduate student at the Scripps Research Kellogg School of Science and Technology who was co-first-author on the paper with Joel Schlosburg of the Lichtman lab. This receptor "downregulation" occurred in some brain areas but not others

To confirm this effect, the researchers utilized another experimental mouse model where the gene for MAGL was inactivated. This lifelong genetic disruption of MAGL also resulted in high 2-AG levels as well as a reduced and desensitized CB1 system.

"Because we're seeing downregulation of the whole cannabinoid system and tolerance to the anti-pain effects, it does raise some concern about whether MAGL would be a suitable pain target," says Blankman.

"If you are going to inhibit MAGL, you probably wouldn't want to produce a complete inactivation of the enzyme," Cravatt adds.

By contrast with the 2-AG experiments, chronically boosting anandamide had none of these effects on the CB1 system. Cravatt doesn't yet know why these two molecules have such different impacts when delivered chronically. He notes, however, that anandamide may be produced selectively under stress conditions, and perhaps for that reason is less likely to trigger a brain-wide CB1 downregulation.

"The question of why anandamide and 2-AG have such different effects when given chronically is certainly going to be motivating us from now on," says Cravatt. "But already with this finding and the development of these models we've taken a significant step forward in understanding and being able to manipulate this important neurotransmitter system."

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