Marijuana Receptor Gene Cloned

The cloning may facilitate the development of new analgesics and other drugs, as well as helping neurobiologists understand how the brain works.

FOR 25 YEARS RESEARCHERS have been looking for a natural high: identifying the cellular receptor that binds the active ingredient in marijuana, known as tetrahydrocannabinol (THC). But the trail proved so difficult to follow that some neuroscientists came to doubt the receptor's existence. That they were wrong to doubt has now been proven by a team from the National Institute of Mental Health.

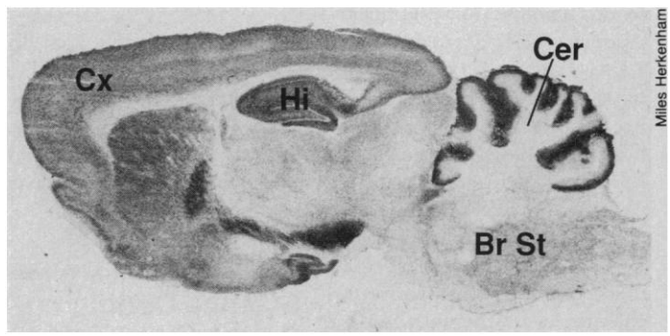
In the 9 August issue of *Nature*, Lisa Matsuda, Stephen Lolait, Michael Brownstein, Alice Young, and Tom Bonner report cloning the gene for the receptor, to which marijuana must bind to produce its effects in the brain. "It's terrific work," says Solomon Snyder of Johns Hopkins University School of Medicine. "It will be a big shot in the arm for both basic research and drug development." Snyder knows a good receptor when he sees one; his group helped identify the brain's opiate receptor in the early 1970s.

The NIMH group's achievement should have important implications for understanding how the brain operates. Since THC isn't normally found in the brain, there must be other, naturally occurring substances that bind the receptor, thereby modulating pain, learning, memory, and other behaviors. Furthermore, the cannabinoids may be useful clinically as analgesics and for treating asthma, glaucoma, and the nausea and vomiting often caused by cancer chemotherapy.

But in spite of its potential importance, the marijuana receptor wasn't the original target of the NIMH team. When they began back in 1987, Matsuda and Bonner were attempting to clone the genes for another group of important receptors, those that bind substance P (see box on p. 625) and neuromedin, peptides that transmit pain impulses in the nervous system. Shigetada Nakanishi of Kyoto University had just cloned the gene for the receptor for substance K, and since it resembles the other two peptides both structurally and functionally, their receptors ought also to be similar, Matsuda and Bonner reasoned.

So they began by making a gene probe, a synthetic DNA sequence that could recognize and bind to a segment of the substance K gene. The probe pulled out a gene all right, and the protein it encoded had the general structural features of a receptor. But it soon became clear, Brownstein says, that it wasn't a receptor for the pain peptides, nor did its structure give any clues about what else it might bind. And that's when the real work began. "It's very easy to clone. You don't even have to go to college," Snyder remarks. "But then what do you do? You have a protein [sequence], but you don't know what it is."

What Matsuda did was to screen a large number of neurotransmitters and hormones for their ability to interact with the protein product of their mystery gene. "We played 'receptor of the week' for about a year and a half," Brownstein says. And all to no avail. The list of compounds screened did not include cannabinoids, he says, because "we had enough to do without looking at long shots." But Matsuda and Lolait did something else that was to prove crucial. They mapped the areas of the rat brain where the gene was active.



Lighting up. *Marijuana receptors dark stain are widely distributed in rat brain. But the pattern is uneven: some areas, including the cerebral cortex Cx, the hippocampus Hi, and the cerebellum Cer, have many, whereas others, such as the brainstem have few.*

Meanwhile, other researchers had been actively pursuing the marijuana receptor, starting back in the mid-1960s when THC was identified as the active ingredient of the drug. But they had run into problems.

The standard way of searching for a receptor is to use a radiolabeled version of one of the compounds that is supposed to bind specifically to it. THC proved unsatisfactory as a probe for the marijuana receptor, however, because it sticks nonspecifically to everything in sight—including cell membranes. That gave rise to the idea that THC might not need to bind a specific receptor, but might yield its effects simply by dissolving in and perturbing cell membranes, as some anesthetics are known to do.

In the mid-1980s, however, Allyn Howlett and her colleagues at St. Louis Unversity Medical School obtained results indicating that cannabinoids do have a specific receptor. Many receptors relay their signals to the cell interior by changing cellular cyclic AMP (cyclic adenosine monophosphate) concentrations, and the St. Louis group showed that cannabinoids inhibit adenylate cyclase, the enzyme that synthesizes cyclic AMP, in cultured nerve cells—a finding that strongly implied that the drugs were not just dissolving nonspecifically in membranes. After eliminating all the known receptors that act by inhibiting adenylate cyclase as a cause of the change they were seeing, the researchers concluded that the cannabinoids were acting through their own receptor.

Further confirmation came from Howlett's collaboration with M. Ross Johnson and Lawrence Melvin of Pfizer Central Research in Groton, Connecticut, who had synthesized a series of cannabinoids with different potencies. Howlett showed that the degree of adenylate cyclase inhibition each compound causes correlates with its potency. That was another sign that there is a cannabinoid receptor, since a correlation of that nature wouldn't be expected for a nonspecific membrane effect. The researchers then used one of the very potent synthetic cannabinoids to characterize the properties of the marijuana receptor in whole rat brains, as opposed to cultured cells.

And that opened the door to solving the identity of the mystery receptor gene. Miles Herkenham, whose lab is just down the hall from Brownstein's at NIMH, and his col-

leagues at NIMH took that same high-potency cannibinoid and used it to map the distribution of the marijuana receptor in rat, guinea pig, dog, and monkey brains, as well as in human brains obtained from people who had died of neurological diseases. The localization of the receptor proved to fit nicely, Herkenham says, with what's known about cannabinoid action.

Marijuana has little effect on respiratory and cardiac function, for example, and the brainstem, which contains the main control centers for those activities, has few of the receptors. But movement control centers, such as the cerebellum, are rich in the receptors, a finding consistent with the drug's ability to cause uncoordinated movements. The receptors are also plentiful in the cerebral cortex and hippocampus, areas important for cognition and memory.

In fact, the receptor is generally so abundant, Herkenham says, that "I realized that the gene was going to come out of some screen someday." He was right.

By the beginning of last year, Matsuda was beginning to get suspicious that her group's mystery receptor might bind cannabinoids. She had found, for example, that the receptor gene was active in the same cell line that Howlett's group had used in much

Substance P Causes Pain—But Also Heals

The National Institute of Mental Health researchers who cloned the gene for the marijuana receptor didn't start out looking for that gene. In fact, they began by looking for genes that encode receptors for peptides known to transmit pain signals in the nervous system, among them the peptide known as substance P.

Found in many organ systems, including the central nervous system, the skin, the lungs, and the intestines, substance P is one of three mammalian neuropeptides known as tachykinins. Its functions are remarkably diverse: in addition to a role in pain, substance P is involved in causing arthritis, psoriasis, and asthma. And some scientists have begun to think that substance P may be a key player in one of the hottest research areas around: links between the immune system and the nervous system.

Paying homage to the varied aspects of substance P, 200 researchers showed up at a recent conference devoted solely to it and its relatives. The conference was put together by Susan Leeman, a University of Massachusetts Medical Center physiologist, who set the field in motion in 1970 by purifying and sequencing the 11–amino acid neuropeptide when she was at Brandeis University.

The best known of substance P's many faces is pain transmission. Noxious stimuli such as heat, pressure, and caustic chemicals, so the theory goes, cause thinly myelinated or nonmyelinated neurons to fire, releasing substance P. Physiologist James L. Henry of McGill University says that substance P then shuts down potassium channels, exciting the nerve cells and making them more responsive.

Experimentally increasing substance P levels in a rat, for example, makes the animal flick its tail sooner in reponse to heat. But, in a surprise twist, substance P also increases cell sensitivity to the body's natural pain killers, such as the enkephalins and the adenosine-mediated neurotransmitters. "What substance P is doing is making the cell more responsive to *any* stimulus that comes along, whether it's a pain input or an analgesic one," says Henry. "That way it's ready for anything."

Substance P also has counterbalancing roles in inflammation: promoting inflammatory processes and perhaps also recruiting cells that heal the damaged area. Neurophysiologist Patrick Mantyh of Veterans Hospital in Minneapolis showed conference attendees that inflamed bowel tissue from patients with Crohn's disease or ulcerative colitis have very large numbers of substance P receptors around blood and lymph vessels; normal tissue has almost none. Similar increases are observed in inflamed joint tissues of rheumatoid arthritis patients and lung tissues of those suffering from asthma.

Likewise, in astrocytes (nervous system cells that are packed in among neurons but don't transmit signals) responding to injury, the population of substance P receptors increases greatly. Ordinarily, astrocytes have few receptors for the neuropeptide. But if a nerve is crushed or damaged, the reactive astrocytes that are nearby "light up like crazy with substance P receptors," Mantyh explained.

This response prevents axons from regenerating—and if a way could be found to block substance P binding, then perhaps central nervous system injuries could be healed by regeneration. In May a group at the Yale University Medical School led by epidemiologist Michael B. Bracken showed that injections of the steroid methylprednisolone within 8 hours of spinal cord injury increase the chance of recovery; the steroid may play some role in blocking substance P binding to its receptor.

Substance P affects inflammation by dilating blood vessels and increasing their permeability and also by stimulating the proliferation of T lymphocytes. It can also recruit leukocytes into inflamed tissue by stimulating them to produce endothelial-leukocyte adhesion molecules, as shown in recent work by dermatologist George Murphy and his colleagues at the University of Pennsylvania School of Medicine.

That ability to "converse with" immune cells suggests a role for substance P well outside the nervous system. "We're increasingly recognizing that the nervous system and the immune system talk to each other," says biologist G. Miller Jonakait of Rutgers University. "And one of the communications links has got to be substance P." She found that a factor produced by white blood cells called interleukin-1 increases transcription of the substance P messenger RNA by injured sympathetic nervous system ganglia—thereby completing an "inflammatory loop" between leukocytes and the neurons that make substance P.

Understanding this loop may have commercial consequences. Many pharmaceutical companies are looking for ways to cut the loop and prevent substance P from transmitting its signals—actions that could promote healing or dampen pain. That task has been made easier by the cloning of the substance P receptor, a task carried out recently by James E. Krause and Andrew Hershey of the Washington University School of Medicine as well as by Shigetada Nakanishi of Kyoto University. Knowing the shape of the receptor will make it possible to focus the search for blocks.

The vast potential for applying the tools of molecular biology to uncover intricate interactions between the nervous system and the immune system—in this case, as mediated by neuropeptides—had conference attendees buzzing, but it also left them with a sense of enormous anticipation. "This meeting has left people dangling," says McGill's Henry, "waiting to see what will happen next." ■ **P. J. SKERRETT**

P. J. Skerrett is a free-lance writer based in Boston.

of its work. So when Matsuda learned about Herkenham's marijuana receptor map, she walked down the hall to see him. They compared maps—and got a flash. The marijuana receptor concentrations were high in just those brain sections where the Brownstein group's gene was active.

She and her colleagues immediately went to work to test the hypothesis that their gene encodes the receptor. They introduced the cloned gene, for example, into Chinese hamster ovary cells and tested the ability of cannabinoids with varying potencies to inhibit adenylate cyclase in the cells. Only cells carrying the transferred receptor gene responded, and then, Brownstein says, "The rank order of the the compounds [in inhibiting the enzyme] was the same as the rank order of those compounds in producing highs." That and other results convinced the researchers that their long search for a function for their gene had paid off.

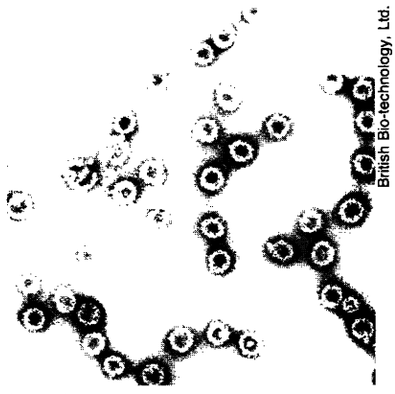
The next big step is to try to find out what the receptor binds naturally. And that could require another protracted search. "We don't even know the chemical nature of the ligand [binding substance]," Brownstein says. "It could be a prostaglandin, a peptide, whatever." Neurobiologists, it turns out, can't expect the instant gratification of marijuana smokers. They have to work long and hard for their highs.

■ JEAN MARX

U.K. Vaccine Trial: Stalking Horse for the Future

The trials of a new AIDS vaccine are set to begin in London next month. And the world will be watching—not just to see how well the vaccine does, but because the protocol will, in several respects, presage future AIDS vaccine trials.

The phase I trials, first under the U.K. Medical Research Council's AIDS Directed Programme, are innovative in three ways. Clinically, the vaccine could prove more effective as a form of immune therapy than as a preventative. Ethically, issues that will arise in later vaccine trials are being given careful consideration. And scientifically, the vaccine's delivery system may be the forerunner of a whole generation of new antiviral immunizations.



Special delivery. *These "virus-like particles" deliver an experimental AIDS vaccine.*

That delivery system consists of virus-like particles, or VLPs. VLPs are made up of proteins and are of the same size and structure as viruses, but contain no DNA or RNA and are therefore not infective. The particles can be engineered to include foreign proteins, serving as the vehicle for presenting antigens—including HIV antigens—to the human immune system.

The first step toward using VLPs as a delivery system was taken in 1984 by Alan Kingsman, a biochemist at Oxford University, who discovered that yeast cells have a gene—named Ty—whose protein has remarkable properties. Ty is a transposon: a piece of DNA that can insert itself in many different spots in the genome. Transposons were already known, but Kingsman and his wife Susan, also an Oxford biochemist, showed that Ty is no ordinary member of that group. Ty is a retrotransposon: like a retrovirus, it reproduces itself via an RNA intermediary and the enzyme reverse transcriptase.

Kingsman went on to show that if he fused another gene to Ty, the yeast cell would make a hybrid protein that assembles itself into VLPs, carrying on their surface antigens corresponding to the foreign gene. And that makes VLPs excellent immunization vehicles. As Kingsman told *Science*, "producing antigens as particles makes them significantly more immunogenic than [they are as] globular proteins."

The Kingsmans' work on VLPs was patented by British Biotechnology, Ltd. Working part time at the firm, where he is associate director of research, Kingsman and his colleagues created hybrid VLPs carrying the p24 protein that forms part of the HIV core. The HIV hybrid has already shown promise—one reason it is proceeding to human trials.

The trials will be conducted by Jonathan Weber, infectious disease specialist at the Royal Postgraduate Medical School in Hammersmith, London. Weber is assembling a panel of 20 healthy young men, not infected with HIV and not in any risk group. The initial phase of the trial is to determine what kind of immune response the VLPs elicit, not to test it against actual infection.

The trial group has taken precautions to ensure that none of the volunteers might be thought to have HIV infection—which could subject them to discrimination. The volunteers will have a positive response to p24—one thing measured by AIDS diagnostic tests. But they will also have a positive response to the Ty protein, distinguishing them from HIV carriers. And, since no HIV envelope protein is included in the vaccine, the volunteers "will absolutely not have an anti-envelope response," Kingsman says. Therefore volunteers in the trial should have no difficulty obtaining life insurance. But such questions will be more sharply posed in the future—particularly if a vaccine containing HIV DNA should ever be tested.

Most workers on AIDS vaccines are cautious about the value of VLP-p24 as a preventative. "The approach is scientifically very interesting," said Reinhard Kurth, director of the Paul Ehrlich Institute near Frankfurt in Germany, "but from the immunological point of view I have my doubts that such a small [antigen] can confer protection." Weber agrees. But, he adds, an effective vaccine may require structural proteins like p24 as well as HIV envelope proteins. Hence the current round of tests might lead to one component of an eventual vaccine.

More promising is use of VLP-p24 as a form of immune therapy. As Kingsman says, "there is a long-standing observation that patients with high anti-p24 levels early in infection tend to have longer onset times." That implies that boosting the level of anti-p24 antigen through the VLPs might delay full-blown AIDS. Kingsman admits that the link between high anti-p24 and delay of AIDS is "controversial." But, he says, it's "worth a try. Anything in the AIDS field is worth a try."

Trying VLPs is worthwhile for another reason—because they can deliver practially any antigen. Hence they might, according to Keith McCullagh, chief executive of British Bio-technology, have "the potential of leading to the creation of a new generation of antiviral vaccines." As one example, Kingsman sees a "much cheaper hepatitis vaccine." And the promise, he thinks, is unlimited: "We can put anything in [VLPs]." ■ JEREMY CHERFAS