PHARMACOLOGY

New Nonopioid Painkiller Shows Promise in Animal Tests

Although morphine has reigned for centuries as the king of painkillers, its rule hasn't been totally benign. Worries about its addictive properties and side effects, such as respiratory depression, have caused many doctors and patients to shy away from it. But a lowly frog could end up threatening morphine's reign. By starting with a toxin found in that animal's skin, researchers have produced a potential new painkiller that works by a different mechanism than morphine and may thus lack some of the opioid's drawbacks.

On page 77, a research team including neuropharmacologist Stephen Arneric, behavioral pharmacologist Michael Decker, and chemist Mark Holladay of Abbott Laboratories in Abbott Park, Illinois, reports promising results in animal tests with a new drug called ABT-594. The researchers found that the drug, which apparently acts not through opioid receptors but through a receptor for the neurotransmitter acetylcholine, blocks both acute and chronic pain in rats. What's more, the researchers have seen few signs that ABT-594 is addictive or toxic in animals.

Much more work will be needed to determine whether the drug is safe and effective in humans, but Abbott has already started safety trials in Europe and hopes to conduct trials in this country as well. "If it works in people, it's going to be a completely new kind of pain reliever," says Howard Fields, professor of physiology and neurology at the University of California, San Francisco.

Such drugs are urgently needed, especially for chronic pain, says pharmacologist Edgar Iwamoto of the University of Kentucky College of Medicine in Lexington. He notes that "30 [million] to 40 million people in the United States have moderate to severe pain that ibuprofen and aspirin just can't handle." And besides many people not wanting to use opioids, "they don't work that well for chronic pain," he says.

Researchers got their first inkling that they might be able to block pain by targeting the acetylcholine receptor in 1932, when they found that nicotine, which binds to one variant of the receptor, dampens pain. The finding lay dormant for decades, however, largely because nicotine is a weak analgesic and causes serious side effects. The field didn't wake up until the mid-1990s, when a compound originally identified in frog skin by chemist John Daly of the National Institute of Diabetes and Digestive and Kidney Diseases burst into the limelight.

In 1976, Daly injected mice with an extract from the skin of a frog he had collected in Ecuador and found that the animal's tail stood up and arched over its back. This intrigued Daly, because he knew that many opioids induce a similar response. "I still remember looking at those mice and getting so excited," Daly says.

Even more exciting, the material, which he called epibatidine after the frog, *Epipedobates tricolor*, was 200 times more potent than morphine at blocking pain in animals. And epibatidine remained effective even when he added chemicals that inhibit opioid action, an indication that it worked through a different set of receptors.

Daly's efforts to isolate and characterize epibatidine were stymied, however, when lab-grown frogs turned out not to make the compound and he could no longer collect the frog in the wild because it had landed on the threatened species list. Daly stored his irreplaceable sample in the freezer and waited, hoping that technology would eventually become powerful enough to tell him what the mysterious compound looked like.

That didn't happen until about 10 years later, when Daly lab members Thomas Spande and Martin Garraffo used nuclear magnetic resonance spectroscopy to determine epibatidine's structure. It resembles that of nicotine: Both have a pyridine ring attached to another ring that contains an amine group. Once the structure was known, several research groups jumped into the fray and synthesized epibatidine. Daly's group went on to show that epibatidine activates the nicotinic acetylcholine receptor.

But while epibatidine is a potent analgesic, it is too toxic for human use. In lab animals, it causes seizures and even death. By then, the Abbott team was also interested, however. They had noticed that it resembles drugs, also aimed at the nicotinic receptor, that the company was studying in hopes of developing treatments for Alzheimer's disease. So the researchers fiddled with their compounds, trying to create a derivative that exclusively kills pain. Out of some 500 variants they produced and then screened in animals, the company decided to focus on ABT-594 because it seemed to work against different types of pain and produce few side effects.

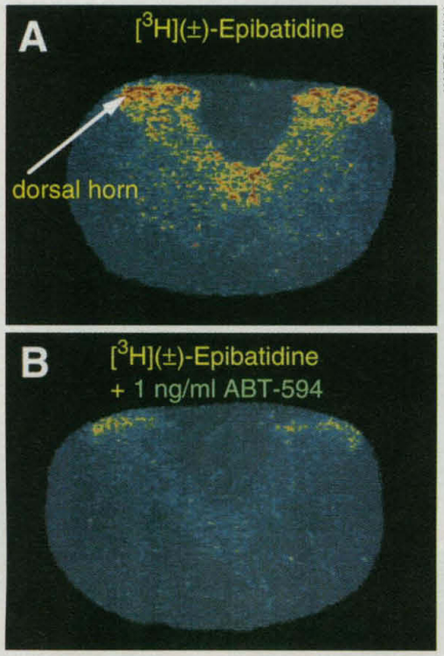
CHARLES W. MYERS/AMERICAN MUSEUM OF NATURAL HISTORY, NYC

Help from a friend. A compound from the skin of the frog *E. tricolor* led to the new painkiller.

As the team now reports, ABT-594 was as effective as morphine in dampening pain from stimuli such as heat or stinging chemicals in rats: The team also mimicked a type of chronic pain in which nerve damage predisposes to pain from stimuli that don't normally hurt. To do this, they surgically compressed a nerve in the spinal cord and then applied different amounts of pressure to the animal's paw. ABT-594 dulls this type of pain as well as morphine does, they found.

At the same time, ABT-594 appears to spare other functions. By placing electrodes in the spinal cords of rats, the researchers showed that the drug hinders the ability of nerve cells to fire in response to harmful mechanical and thermal stimuli, but it does not affect responses to benign sensations such as touch or mild heat. The company also found, Arneric says, that ABT-594 depresses the respiratory system much less than morphine does and makes animals more alert instead of sedating them.

ABT-594 may be less toxic than epibatidine because it has more selective effects on neurons. Tests on membranes containing acetylcholine receptors showed that ABT-594



JAMES PAULY

Blocked out. Epibatidine binding (yellow to red color) in the spinal cord is reduced with ABT-594 present *(bottom)*, an indication that both compounds bind the same receptors.

binds several hundred thousand times more tightly to a nicotinic receptor from the central nervous system, where neurons process pain information, than to one that tells muscles to contract. For epibatidine, that ratio was only 57 to 1. "They came upon a compound that gets rid of the toxic effects of epibatidine and still has analgesic capabilities," says Daly. "I would not have thought it possible."

And in at least one test, ABT-594 appeared to be nonaddictive. Rats that were taken off ABT-594 after being treated with a high dose for 10 days did not suffer the withdrawal symptom of appetite suppression seen after treatment with opioids. Other researchers point out, however, that ABT-594's mechanism of action raises the possibility that it will lead to other forms of dependency. "The company would hope that their drug isn't addicting because it doesn't act through the opioid receptor," says Fields. "But nicotine is addictive, too."

The big question now is whether this early promise will be borne out when the compound is tested in humans. An indication ought to come in a few months when the first results from the European safety trials become available. "We're crossing our fingers and anxiously looking forward to the summer," says Michael Williams, Abbott Labs vice president of neurological and urological drug discovery.

–Evelyn Strauss

Evelyn Strauss is a free-lance writer in San Francisco.

IMAGING

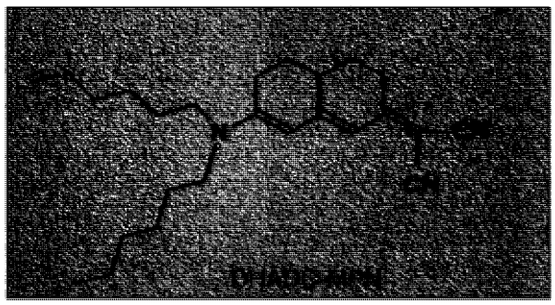
Putting the Infrared Heat on X-rays

Doctors would love to put an old workhorse—x-rays—out to pasture. Although x-rays are still an indispensable tool for diagnosing everything from broken pinkies to lung tumors, the energetic beams can damage DNA, posing a slight cancer risk. Infrared light, which can pass through tissue harmlessly, could bring a softer touch to medical imaging. But to turn it into a serious rival for x-rays, researchers need a simple way to compose an image from the few infrared photons that pass directly through tissue without getting scattered. In this issue of *Science*, a team from the University of Arizona in Tucson and the California Institute of Technology (Caltech) in Pasadena reports about a light-sensitive polymer that might do the job.

The polymer, described on page 54, can change its optical properties in response to a subtle play of light: the interference between the few infrared photons traveling straight through a scattering medium and a separate infrared beam. The result is a pattern called a hologram, from which a three-dimensional (3D) image of the tissue can be reconstructed. Until now, separating the few straight-shooting photons from the scattered stragglers to make an image has required unwieldy gas-, liquid-, or crystal-based detectors, but the new polymers are easy to handle and cheap to process into film. "They could be very useful," says Robert Alfano, an imaging researcher at the City College of New York. But experts acknowledge that infrared systems are a long way from the clinic, because these systems so far can only image thin tissue slices.

For years, researchers have been tinkering with polymers that store holograms made by visible light. Storing an image requires relatively simple physics: Two laser beams—one carrying information about the image to be stored and the other a "reference" beam—intersect in the polymer, setting up an interference pattern of bright and dark areas. "Sensitizing" compounds in the polymer's lit regions absorb photons, which excite electrons to a higher energy level. Electrons from a surrounding matrix of charge-conducting molecules rush to fill the gaps in the electron shells, resulting in transient positive charges that ripple through the matrix until getting trapped in the dark region. The light-dark interference pattern thus is reproduced in the polymer as a pattern of corresponding positive and negative charges.

These islands of charge attract a third class of compounds in the polymer: dye. The stringy dye molecules themselves are polarized, having opposite charges on either end. The positive end swivels toward a cluster of negative charge, and vice versa. This reorientation alters the polymer's index of refraction, or the speed at which light moves through the film. The 3D pattern of varying refractive index is a hologram. As long as the charges remain fixed in one of these photorefractive polymers, a hologram persists. When lit up by another laser beam, the polymer diffracts the photons in a pattern, reproducing the original image.



KIPPELEN *ET AL.*

Seeing the IR light. New dye molecule may help usher holographic imaging into the medical lab.

The research team, led by Arizona's Bernard Kippelen and Nassar Peyghambarian, and Caltech's Seth Marder, realized that such holograms might be useful for medical imaging. Most photorefractive polymers, however, are sensitive to visible light, which tissues readily absorb or reflect. To exploit the near-infrared light that is best for probing tissue, Kippelen and his colleagues added a new sensitizer to standard polymers that releases positive and negative charges after absorbing infrared photons.

This modification alone was not enough to make the polymer useful for medical imaging. The researchers also had to boost the polymers' signal-to-noise ratio to record the few photons that make it through tissue. To do so, they created new dye molecules that are adept at orienting their charged ends in the weaker electric fields created by fewer incoming photons. The souped-up polymer had the same sensitivity as the best visible-light photorefractive polymers, while receiving four times fewer photons.

Next, Kippelen's group set out to reproduce the image of the number "5" in a photorefractive film. First, they generated an infrared laser pulse and split it into two separate beams. One beam passed through a transparent "5" in an otherwise opaque sheet of photographic film. Photons emerging from the "5" passed through polystyrene beads floating in an organic solvent, a scattering medium used to simulate human tissue, before impinging on the polymer. Inside the polymer, the first wave of photons—which had emerged unscattered—crossed paths with others from the second beam that had been routed around the barriers and timed to arrive simultaneously. As photons from the two beams interfered, they reproduced the "5" as a hologram that could be read by another infrared beam. Scattered photons arrived too late to set up an interference pattern with the second beam; thus they were unable to muddy the hologram.

Similar feats have been accomplished using cesium vapor and other materials as the holographic storage medium, says Irving Bigio, a holographic storage expert at Los Alamos National Laboratory in New Mexico. But the new polymer films, he says, "look far easier to use." Major obstacles remain, however, before these new photoreactive polymers appear at the doctor's office. The key hurdle, Bigio says, is that no infrared technology designed so far can image tissue thicker than about 1 centimeter. The hunt is now on for new schemes to boost the number of usable photons, or make the most of the ones that get through. Until these efforts pay off, however, x-rays will remain a radiologist's best friend.

–Robert F. Service