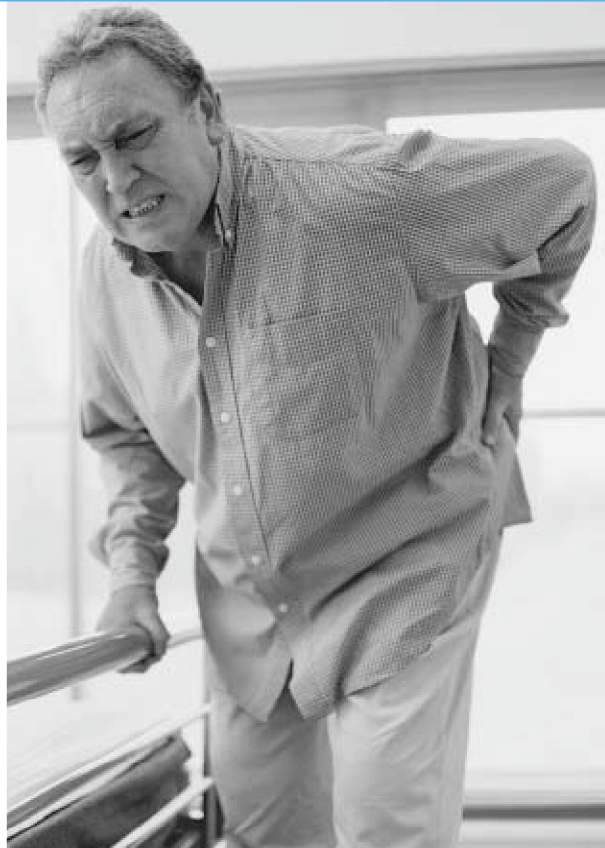


PAIN



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Medical researchers devoutly seek better ways to control pain. Toward that goal, pain researchers take many approaches, and while their achievements are encouraging, the very diversity of their findings reminds us how complex and multifaceted pain is. Last year's notable achievements reinforce the point. One finding suggests how to prevent morphine overdoses from causing suppression of breathing; another reveals a connection between pain, the immune system, and a type of receptor connected with marijuana; a third demonstrates a novel scheme for enlisting gene therapy to help control pain. And certainly no one expected this revelation in 2003: a link between pain, gender, and, of all things, red hair.

Opioids Without Their Downside: Breathing Suppression

Opioids are excellent analgesics but carry the risk of slowing breathing or even stopping it entirely. Neuroscientist Diethelm Richter of Germany's University of Göttingen set out to see if he could separate the analgesic effects of the commonly used opioid drug fentanyl from potentially dangerous respiratory depression, believed to be responsible for many deaths during the rescue of hostages at a Moscow theater in 2002.

He and his team began by examining a small area in the rat brainstem responsible for generating the neural activity of breathing, the pre-Botzinger complex, or PBC. There they found serotonin receptors—the endogenous neurotransmitter serotonin is known to influence the activity of the breathing center—and mu-opioid receptors, which were assumed to interact with the mu-opioid pain pathway. They discovered that using an agonist to activate one subtype of serotonin receptor, 5-HT4(a), overcame fentanyl-induced respiratory depression without diminishing fentanyl's ability to dull pain.

In these experiments, they treated rats with the 5-HT4(a) agonist BIMU8. They began by verifying that fentanyl-activated mu-opioid receptors produced analgesia (as seen by block of

the tail flick, a common pain test in which a heat stimulus is applied to a rat's tail) and also suppressed respiration. Then they asked if activating the 5-HT₄(a) receptor would overcome fentanyl-induced breathing suppression. It did: Giving the rats BIMU8 after fentanyl re-established stable breathing activity. They then studied whether treating with BIMU8 after administering fentanyl destroyed the pain relief response and found that it did not.

Their experiments, published in July in *Science*,⁶³ show that it is possible to fine-tune the effects of an opioid analgesic. Richter believes that selective 5-HT₄(a) serotonin agonists developed for human use could restore breathing in instances of opioid overdose and protect chronic pain patients who take high doses of opioids from respiratory depression. 5-HT₄(a) agonists might also restore patients to spontaneous breathing following surgery performed under opioid anesthesia.

Stimulating CB2 Cannabinoid Receptors Relieves Neuropathic Pain

CB1 receptors are the cannabinoid receptors inside the central nervous system (CNS); outside, CB2 receptors are located on peripheral immune cells and mast cells. THC, the active ingredient in *Cannabis sativa*, or marijuana, stimulates both receptor types; its famous effects of sedation and euphoria come from stimulating CB1 receptors in the CNS.

Neuropathic pain, associated with injuries to peripheral nerves such as can occur in diabetes, affects about 1 to 2 percent of the population. Despite that pervasiveness, effective treatment has been elusive. Existing drugs for neuropathic pain work through the CNS and can cause undesirable side effects such as dizziness and sleepiness. At the University of Arizona, Philip Malan knew that targeting cannabinoid receptors diminishes neuropathic pain but sometimes causes undesirable CNS effects. He thought a drug acting on pain receptors outside the CNS might avoid those side effects.

In studies published in August in the *Proceedings of the National Academy of Sciences*,⁶⁴ Malan and his team tested a pain-killing drug called AM1241, designed and synthesized by

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Alex Makriyannis of the University of Connecticut, in an experimental mouse model of neuropathic pain. They found that in mice AM1241 reversed surgically induced neuropathic pain. Following up, they noted that a CB2-receptor antagonist blocked pain relief with AM1241 while a CB1-receptor antagonist did not. This indicated that CB2 receptors mediate analgesia produced by AM1241.

While they do not understand exactly how AM1241 works, they hypothesize that its activation of CB2 receptors decreases the sensitivity of primary afferent neurons by inhibiting release of pain-sensitizing substances from surrounding mast and immune cells. Notably, even though the CB2 receptor is found on immune cells, they have not seen evidence that AM1241 inhibits immune function.

Malan tested AM1241 against two other types of pain— inflammatory and nociceptive—and found it was effective there as well. (Pain and other signs of inflammation such as redness, swelling, and warmth are caused by biochemical reactions within blood vessels in the vicinity of injured tissues. Nociceptive pain, typically a dull ache, results from disease or injury outside the nervous system—in contrast to the neuropathic pain of damaged nerve tissues.) Malan observes that many pain conditions such as cancer pain combine more than one pain type. An analgesic like AM1241 that is effective against neuropathic and other types of pain could be a huge advance if it tests well in humans. Meanwhile, Malan and colleagues plan to test AM1241 in additional animal models of pain such as visceral pain seen in inflammatory bowel disease.

Gene Therapy Approach to Treating Pain

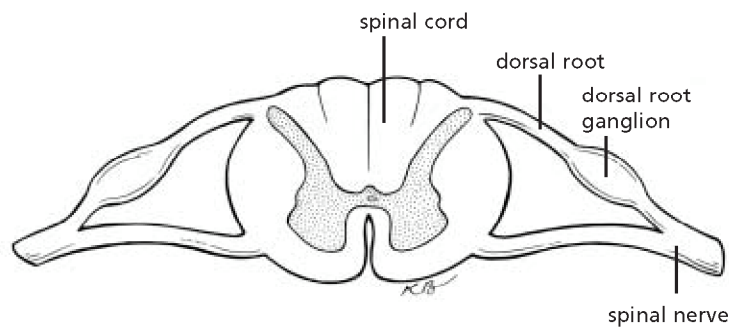
A gene therapy approach to treating pain might enable patients to take smaller amounts of opioids, which might in turn produce fewer side effects, last longer, and reduce the chance of

inducing tolerance. In research published in May in the *Proceedings of the National Academy of Sciences*,⁶⁵ Li-Yen Mae Huang and her colleagues at the University of Texas Medical Branch in Galveston have taken a step in that direction.

Previous gene therapy experiments in animals by other researchers delivered precursor genes for opioid peptides and produced pain relief for up to eight weeks—but also some toxicity. Huang and her team hoped to improve on both side effects and efficacy. Rather than providing analgesia directly with precursor genes, her idea was that increasing the number of receptors for mu-opioids might enable the body to experience pain relief at lower opioid doses. As a gene therapy vector, the team chose a recombinant adenoviral-associated virus (rAAV) for its relative low toxicity and ability to provide long-lasting gene expression. By coupling the rAAV gene-therapy vector with the mu-opioid receptor (mOR) gene rather than a gene for a pain-relieving opiate peptide, they hoped to avoid tolerance and respiratory depression.

Using gene therapy, Huang introduced the mOR gene with a neuron-specific promoter directly into the dorsal root ganglia (DRG) in rats. DRG pre-synaptic neurons carry pain signals to the brain via the spinal cord's dorsal horn.

To see how the mOR gene affected the rats' pain response, the researchers tested the rats with heat stimulation to the paw.



Gene therapy

Neurons in the dorsal root ganglia (DRG) transport pain signals from the spinal cord to the brain. A type of DRG gene therapy improved the ability of morphine to lessen pain from heat stimulation in rats.

Huang found that mOR gene therapy produced long-term gene expression in DRG neurons, which markedly enhanced the ability of morphine to relieve pain from thermal stimuli.

Huang suggests that gene therapy for pain relief is feasible for humans, but with the modification of gene delivery to the sciatic nerve rather than the DRG because of better accessibility and safety. Such a genetic approach to pain control could benefit patients with chronic cancer pain and other conditions requiring long-term use of opioid drugs.

Gender Differences in Pain and Analgesia Explained

Many physicians have observed differences in how men and women experience pain, noting, for instance, that some analgesics seem to work better in women than in men. In surprising new research, Jeffrey Mogil of Montreal's McGill University identified a gene that controls one of the sex-specific neural mechanisms underlying these differences.

Ten years ago Mogil discovered that male and female mice process pain via two different systems. He found that an experimental drug, MK-801, reversed stress-induced analgesia in male but not female mice. This implied that females had a separate system of pain processing. Subsequently he demonstrated that the female-specific pain-processing system is enabled in an "on-off" manner by circulating estrogen hormones.

In his latest research, published in the April *Proceedings of the National Academy of Sciences*,⁶⁶ he investigated another pain-processing system influenced by gender. He and his team tested sex differences in the kappa opioid class of analgesics, which many reports indicate are effective in females but not

For both types of pain, pain relief with pentazocine was more significant for red-haired and fair-skinned women with two variant MC1R alleles than for any other group.

males. Through gene-mapping studies, they associated kappa-opioid pain processing to the melanocortin-1 receptor (MC1R) gene in mice on chromosome 8. This receptor was already well known, but in an entirely different context: It influences hair and skin color in humans and coat color in mice.

Mogil's team discovered that MC1R mediates kappa-opioid pain control only in females. They tested male and female mice with pentazocine, a kappa opioid that acts upon ischemic and thermal pain. MC1R-gene variants influenced pain relief with pentazocine, but only for females.

They found a similar effect for humans. For both types of pain, pain relief with pentazocine was more significant for red-haired and fair-skinned women with two variant MC1R alleles than for any other group. More generally, Mogil's work shows the potential power of pharmacogenetics: how patients' genetic information might help physicians decide which drugs to prescribe. And by the same logic, knowing how pain-control circuitry works at the genetic level may help scientists develop drugs that work better in particular populations.