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Mouse Study Points to Understanding, Enhancing Pain Therapy

Knocking out a gene that muffles the painkilling signal of morphine yields animals that can tolerate pain for longer periods of time, even while receiving lower doses of the drug, reports a team of HHMI investigators.

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— **Robert J. Lefkowitz**

Robert J. Lefkowitz and Marc G. Caron, whose laboratories are at Duke University Medical Center, plan to use their genetically engineered mice to try to uncover long-sought answers about how the commonly prescribed painkiller morphine builds tolerance and sometimes dependence in patients. Those discoveries, in turn, may lead to new drugs that can enhance morphine's medicinal benefits while reducing its side effects.

"For the first time, we have a tool to ask whether the mechanisms that we know desensitize the opioid (morphine-binding) receptors are related to the rise of tolerance, physical dependence, and even addiction," explained Caron.

The engineered mice developed by Fang-Tsyr Lin and Karsten Peppel in Lefkowitz's laboratory appear normal but lack a gene encoding a protein called beta-arrestin 2. The protein normally rests in the cell's cytoplasm. But when it senses that a receptor on the cell membrane is activated, it binds to the receptor and temporarily blocks any further incoming signal. This action protects the cell from overreacting to a signal whether to grow, release a hormone to speed up the heart, produce an enzyme to digest food, or a host of other actions.

When it comes to alleviating pain after surgery or in patients with cancer, however, the switch may work too efficiently. Lefkowitz and Caron decided

to test whether beta-arrestin 2 was the switch that blocked opioid receptors, thus turning off morphine's analgesic (pain-reducing) signal.

Laura Bohn and Raul Gainetdinov of Caron's team designed experiments using Lin's knockout mice. Bohn injected both knockout and normal, or wild-type, mice with morphine and placed them on a mildly uncomfortable warm surface for up to 30 seconds. She then timed how long the animals stayed before licking or flicking their paws.

"Lo and behold, the difference was night and day," said Lefkowitz. "The knockout mice had a much more prolonged, much greater response to morphine. In other words, the receptors didn't turn off."

In a research article published in the December 24, 1999, issue of the journal *Science*, the researchers describe how the knockout mice exhibited significant analgesia (about one-third their peak response) four hours after receiving their morphine dose. In contrast, wild-type mice were back to baseline in about 90 minutes.

"We also found that the knockout mice were much more sensitive to the morphine," added Caron. "They could achieve the same amounts of analgesia with about 10 times less drug."

To confirm that the opioid receptors were responsible for the mice's pain reduction, Bohn treated them with naloxone, a drug that displaces morphine from the receptors. Both knockout and wild-type mice lost the analgesic effects within 10 minutes. The researchers also conducted a variety of biochemical experiments to further confirm their findings.

"Our results suggest that if we had a drug that could safely inhibit beta-arrestin 2, patients could benefit from longer pain relief on a lower dose of morphine," said Lefkowitz. Morphine, while currently the analgesic of choice in cases of severe or chronic pain, is not without side effects: Constipation, nausea, drowsiness, and breathing suppression can develop as well as tolerance and physical dependence.

"And now we have the means to answer some very fundamental questions in narcotics research," Lefkowitz continued. "Say we take mice and inject them daily with morphine for a week. The normal mice would become tolerant and physically dependent, but what's going to happen to the knockout mice? Can they get tolerant? Physically dependent? People would love to know. And within months, we'll have the answers."

Lefkowitz and Caron suspect that regulation of other receptors there are likely hundreds of kinds that interact with beta-arrestin 2 is altered as well in the knockout mice. "So although we're excited about the morphine angle, we have plenty of other experiments to perform as well," said Lefkowitz.

Lefkowitz, who specializes in regulation of the cardiovascular system, and Caron, particularly interested in the neurological pathways of addiction, have collaborated for nearly 25 years in signal receptor research.