High-powered X-ray laser unlocks mechanics of pain relief without addiction

February 18 2015

An ampoule of morphine with integral needle for immediate use. From WWII. On display at the Army Medical Services Museum. Credit: Gaius Cornelius/Wikipedia

Using a newly developed X-ray source, scientists have revealed how a new type of pain-relievers works - bonding to the same neuroreceptors that morphine does, but without the accompanying physical dependence.

"If you know how the binding physically works, you can design molecules to target the specific receptor sites and generate specific responses," said Vadim Cherezov, professor at the USC Dornsife College of Letters, Arts and Sciences and corresponding author on a paper about the research that was published online by *Nature Structural and Molecular Biology* on Feb. 16.

Opioid receptors throughout the brain and spinal cord exist in four major subtypes: delta, kappa, mu, and Nociceptin receptor. The body naturally releases molecules, or "ligands," called enkephalins, endorphins and dynorphins that bind to these receptors, regulating mood and pain.

Opiate drugs like morphine try to artificially do the same thing, targeting mu receptors. While they effectively relieve pain by doing so, their prolonged use causes a growing tolerance to the drug and, ultimately, physical dependence.

Previous research has shown that administering morphine with a chemical that simultaneously blocks delta receptors cuts down on those negative side effects - leading researchers to believe that there must be a way to engineer a drug that interacts with both the mu and delta receptors in such a way that it would have all of the benefits of morphine but none of the drawbacks.

To that end, Cherezov and his team focused on a molecule that is structurally similar to the body's natural ligands, but operates differently - activating mu receptors while blocking delta receptors.

The only problem - these receptors are incredibly difficult to image.

"Drug abuse is the leading cause of injury related deaths in the United States and opioid abuse cost the U.S. approximately $58 billion in 2007. The structural data are helping to provide new insight into the

understanding of how the receptors work in our body, and the design of novel molecules that might help address this critical health issue," said Raymond Stevens, co-author of the study and director of the Bridge Institute at USC Dornsife.

To figure out what a tiny biological structure looks like, scientists usually grow crystals of them and then blast those crystals with X-rays; piecing together a picture of what they look like based on the way that the X-rays bounce off of them.

The process, known as X-ray crystallography, works best with rigid biological structures because their rigidity allows them to support the growth of larger crystals. X-ray beams pummel and destroy crystals quickly, and small crystals don't have the fortitude to stand up to them long enough to collect enough data.

The receptors Cherezov wanted to study are highly dynamic and flexible, and typically can't grow such large crystals.

Instead, Cherezov and his colleagues used a new technique that features a brighter X-ray free electron laser. While it might seem counterintuitive, blasting the crystals with a higher-intensity but very short X-ray pulses allows Cherezov to use smaller crystals at near-body temperatures, rather than cryo-cooling them.

"They vaporize in an instant, but before disintegrating they reflect photons producing an image from un-damaged molecules," Cherezov said. "The result is terabytes of data from tens of thousands of crystals, which our colleagues at the Center for Free Electron Laser Science in Hamburg help us to analyze in order to translate them into a single model for the structure."

While traditional crystallography relies on crystals being a bare

minimum of 10 to 20 microns across to last long enough to be useful, the free electron laser allows Cherezov to image crystals that are two orders of magnitude smaller by volume.

The resulting structural model has a resolution of 2.7 angstroms (an angstrom being roughly the length of the radius of an atom), which is precise enough to see how molecules bind with the receptor.

Next, Cherezov and his colleagues plan to use X-ray lasers to study how receptors like these interact with their signaling partners, and to record a molecular movie of how these receptors transmit signals across the membrane.

For this study, Cherezov and Stevens collaborated with colleagues at Scripps Research Institute, Arizona State University, Vrije Universiteit Brussel in Belgium, University of North Carolina Chapel Hill Medical School, SLAC National Accelerator Laboratory, Deutsches Elektronen-Synchrotron in Germany, the University of Hamburg, European XFEL GmbH in Germany, and the Clinical Research Institute of Montreal in Canada.

More information: *Nature Structural and Molecular Biology*, www.nature.com/nsmb/journal/va … /full/nsmb.2965.html

Provided by University of Southern California