Cellular gatekeepers do more than open doors for drugs, study finds

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(Medical Xpress)—The cellular gatekeepers that escort the most common pharmaceuticals into our cells continue to work within the cells as well, according to a UC San Francisco discovery that could transform drug design and lead to new ways to treat disease.

Almost half of approved pharmaceuticals—for cancer, heart failure, inflammatory diseases, and others—act through gatekeepers on cell surfaces known as G-protein-coupled receptors (GPCRs). More than 1,000 specific GPCR proteins play roles in nerve signaling, immune responses, sensory perception and many other physiological phenomena governed by the trillions of cells within our bodies.

A team led by UCSF cell biologist Mark von Zastrow, MD, PhD, now has demonstrated that these receptor proteins can remain active longer than expected—even after being pulled into the cell after the attachment of a drug or natural activator. The researchers also demonstrated the value of a new way to study the receptors once they're inside the cell, one which may shed light on why therapeutics have unexpected effects or varying degrees of effectiveness, according to von Zastrow. The study appeared in the March 28 issue of *Nature*.

"From the standpoint of drug development, this may open a vast new range of targets, involving the cellular machinery that determines whether or not these key receptor molecules are present on the cell surface," said von Zastrow, a cell biologist who holds the Friends of LPPI Endowed Chair for Research in Schizophrenia and Depression at

UCSF.

Using small, genetically engineered "nanobodies"—tiny antibodies similar to those naturally present in camels, but in few other animals—the researchers were able to trace short-lived chemical liaisons between molecules and to demonstrate that a GPCR known as the beta-adrenergic receptor remains active for minutes after it is internalized within the cell. The same receptor often has served as a model for studies of GPCRs, including studies by Brian K. Kobilka, MD, of Stanford University and Robert J. Lefkowitz, MD, of Duke University, who shared the 2012 Nobel Prize in Chemistry for discoveries related to GPCRs.

Many drugs mimic the shapes of natural signaling molecules and block or activate specific GPCRs. When activated, the receptor acts upon another protein—its own specific partner within a class of enzymes known as G proteins. In a chain reaction, G proteins then initiate their own effects downstream in the cell's biochemical pathways.

The small size and flexibility of the engineered nanobodies allow them to attach to parts of proteins that other antibodies can't reach. Von Zastrow's team developed one fluorescent nanobody to bind selectively to the transitional, activated form of the beta adrenergic receptor, and another to bind to the activated form of its G protein. This enabled the researchers to trace the receptors' activity as they moved inside the cell from the cell surface.

Noting that different drugs can have different effects, even when targeting the same receptor, von Zastrow suggests that nanobodies might help drug developers gain insight into whether these differences are caused by attachments to different transient shapes formed by GPCRs. The engineered nanobodies could serve as tools to tag key activated forms of GPCRs as the receptors interact with other molecules.

Nanobodies might even be adaptable for high-throughput drug screening, von Zastrow suggested.

Tracking GPCRs in the cell

UCSF postdoctoral fellow Roshanak Irannejad, PhD, who performed most of the key experiments and was first author on the paper, used isoproterenol—a drug for treating slow heart beat—to activate the beta-adrenergic receptor and trace activity over time and space, on and within the cells of a human kidney cell line.

Von Zastrow is an expert on endosomes, small membranous sacs pinched off from cell membranes and taken into cells. Each cell may contain 1,000 or more endosomes. Different endosomes contain different molecules. GPCRs were thought to be inactive within endosomes, where they awaited one of two fates—being sent to the cell's trash heap, or being recycled to the cell surface. Once activated, GPCRs are taken into the cell via endosomes.

"It was previously thought that once the receptor is taken away from the cell's surface that it no longer does anything until it is recycled back to the surface later," he said. "Our data clearly show that's not true—the internalized receptors actually are active and doing specific things within cells."

Von Zastrow speculates that endosomes might sometimes help redeploy GPCRs to different parts of the cell surface where they are needed, a function that might prove especially valuable to oddly shaped cells such as neurons, which have long, branching processes—axons and dendrites—and large surface areas. Drugs might one day be targeted to manipulate the internalization and movement of specific GPCRs via endosomes, von Zastrow said.

More information: www.nature.com/nature/journal/ … ull/nature12000.html

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