Research on rarely studied cell-receptor regions opens door to eliminating drugs' side effects

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Researchers at the Stanford University School of Medicine have taken an early step toward identifying a new approach to drug discovery that may eventually yield drugs with fewer side effects.

In a study to be published online Jan. 7 in *Nature,* investigators led by senior author Brian Kobilka, MD, professor and chair of molecular and cellular physiology, found that largely neglected regions on key cell-surface proteins undergo minute changes in shape in response to drugs and thus could prove useful in drug design. The study's first author is Michael Bokoch, an MD/PhD student in Kobilka's laboratory.

The class of proteins known as G-protein-coupled receptors, or GPCRs, is already immensely important in drug research, accounting for some 40 percent of all currently marketed drugs, said Kobilka. His laboratory focuses on a particular type of GPCR called adrenergic receptors, which are activated by adrenaline and its close cousin noradrenaline. Secreted by the adrenal glands and certain nerve cells, these two "molecules on a mission" regulate key physiological actions in the central nervous system , heart and musculature. They are acclaimed for tripping off the "fight or flight" response, which steels middle-aged mortals' melting muscles for high-stakes activities like fending off saber-tooth tigers or running to catch a bus.

Like all GPCRs, an adrenergic receptor consists of three portions. One is

anchored within a cell's outer membrane. The second juts from the cell's outer membrane surface and is exposed to the external environment. And the third extends into the cell's fluid interior, or cytoplasm.

Cell-surface receptors are akin to customized doorbells that ring only if pressed by molecular fingers with precisely the right shape. For an adrenergic receptor, the fingers with the magic touch are adrenaline and noradrenaline. When a molecule of one of these structurally similar substances happens upon an adrenergic receptor, it is drawn to a site on the receptor called a binding pocket with just the right shape and charge for a snug fit. (An adrenergic receptor's binding pocket sits within the portion of the receptor that is anchored in the cell's outer membrane.) The binding event alters the shape (or "conformation") of the receptor's cytoplasm-facing domain, setting off a massive redirection of activity inside the cell.

Other molecules besides adrenaline and noradrenaline can slip into adrenergic receptors' binding pockets. That's the basis for many effective drugs. Cell-surface receptors are great targets for the small molecules that drug developers discover and deliver into our bodies to stimulate or shut down a physiological process. Various drugs can affect the same receptor quite differently. "Agonists" lock their targeted receptor into an active or even hyperactive mode. "Antagonists" force the receptor into a sluggish or inactive posture so that it stalls out, or they simply get in the way of the naturally occurring molecules the receptor was meant to match.

Adrenergic receptors come in nine different varieties, or subtypes, all responsive to adrenaline and noradrenaline but playing different roles in regulating bodily functions. For instance, Kobilka said, the beta-2 adrenergic receptor is the chief regulator of smooth muscle, especially in relaxing air passages such as the lungs (a fight-or-flight necessity). This makes beta-2 agonists, which open airways, good for combating an

asthma attack.

It is primarily the beta-1 receptor that accelerates the heartbeat and stimulates the heart to pump more blood per beat. That's also great for a fight-or-flight response. But too much beta-1 stimulation over an extended period can lead to medical problems like heart failure. Thus, beta-1 antagonists (also known as beta-blockers) are often prescribed for patients with coronary artery disease, heart failure or arrhythmias.

The trouble is, drugs that fit in one subtype's binding pocket can often climb into the other's. "The beta-1 and beta-2 receptors largely respond to drugs in the same way. That's one reason we get side effects," said Kobilka. "Say you have a patient with both heart disease and asthma. You want to treat that patient's lung problem with beta-2 agonists. But those may stimulate beta-1 receptors in the heart, potentially causing arrhythmias. So you can't use beta-2 agonists in that patient. Similarly, you may not be able to use a beta-1 antagonist for this patient's heart problem, because it may exacerbate the asthma."

While the binding pockets of various adrenergic-receptor subtypes have to be nearly identical so they can all attract and bind adrenaline and noradrenaline, certain portions of these receptors' exposed outer domains have been freer to diverge over eons of evolution. The extracellular portions of the beta-1 and beta-2 adrenergic receptors, for instance, are quite different. A drug that bound selectively to an extracellular section of one receptor subtype — but not of the second subtype — in a way that altered the receptor's cytoplasmic conformation just as do drugs that target the receptor's binding pocket might be more selective, minimizing side effects.

Kobilka and his colleagues used a sensitive technique called nuclear magnetic resonance spectroscopy to zero in on one specific part of the beta-2 adrenergic receptor's extracellular domain to see if they could

detect subtle changes in that area when they applied three different drugs: a beta-2 adrenergic-receptor agonist, an antagonist and a third one with a neutral effect on the receptor's activation status. Even though the drug molecules themselves landed smack-dab in their binding pockets as expected, each drug coaxed this part of the receptor's extracellular domain into a different conformation. This suggests, said Kobilka, that the conformational shifts of this region were coupled to those triggered by the drugs' interactions with the receptor's binding pocket.

If this coupling works in reverse, molecules that bind to the extracellular domain could conceivably modulate receptor function. Thus, the diversity of different receptors' extracellular domains could be exploited to modulate receptor activity, with high subtype selectivity.

Even if the tail-wags-the-dog effect were only slight, Kobilka said, drugs targeting GPCRs' extracellular surfaces would still let natural molecules fit into their binding pockets. "So, instead of simply switching receptor activity on or off they could instead fine-tune that activity, like rheostats. For therapy, it would be nice to control the receptor's activity in this way."

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