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RESEARCH NEWS

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Structural Studies Reveal How Potassium Channels are Inactivated

Researchers have discovered how potassium channels can slam shut, a control mechanism that allows neurons to regulate their firing frequency.

Using techniques to produce precise mutations in the channels, the researchers then used x-ray crystallography to deduce that one of four long “tails,” called inactivation gates, at the end of the channel can slide into the channel’s pore and shut it down. The studies not only settle the question of how potassium channels manage to close milliseconds after opening, but also offer new insights that will aid in designing drugs that control the channels more precisely.

The research team, led by Howard Hughes Medical Institute investigator Roderick MacKinnon at The Rockefeller University, published its findings in the June 7, 2001, issue of the journal *Nature.*

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- Roderick MacKinnon

To maintain the correct concentration of potassium, cells are equipped with potassium ion channels, pore-like proteins that poke through the cell membrane. These channels create sieves through which potassium ions flow from inside to outside the cell. Moving potassium through the cell membrane is critical to numerous life-sustaining functions, including the beating of the heart, insulin release in response to changes in blood sugar, and nerve signal generation. When a nerve signal travels the length of a neuron, for example, large amounts of potassium must be able to flow quickly from the inside to the outside of a cell. This outflow of potassium allows the membrane to return to its resting state and prepare for the next impulse.

Structural studies conducted during the last three years showed that the channels are composed of four identical subunits that fit together to form a

cone-shaped pore that spans the cell membrane.

In earlier studies, MacKinnon and his colleagues reported that potassium channels have side openings that are located above a portion of the channel that juts into the cell's interior. Those experiments showed that the side windows offered a potential route through which an inactivation gate can reach the central pore of the channel. Inactivation gates are the means by which the channel's control machinery can quickly slam the channel shut to halt the flow of potassium ions.

A question arose about whether the inactivation gate could fit through a side window to plug the pore. According to MacKinnon, the earliest work on ion channels suggested that the pore was plugged by a "ball and chain" mechanism, in which a tethered particle would plug the pore. But it seemed that a particle would have considerable difficulty working its way through the side openings, MacKinnon said.

Studies by HHMI investigator Richard W. Aldrich and colleagues at Stanford University had demonstrated that the N-terminal segment of the channel was involved in inactivation. "Aldrich and his colleagues found that if they removed the N-terminal amino acids, the voltage-dependent gate would open, but the channel would not inactivate," said MacKinnon. "They did very specific point mutations of those amino acids to describe which ones were important for this process."

What remained unclear after Aldrich's studies, said MacKinnon, was where the inactivation gate goes and how it works to plug the channel. MacKinnon and his colleagues hypothesized that the inactivation gate inserts itself deep into the pore to close the channel. They based their theory on the fact that the structure of the potassium channel -- which they solved in 1998 -- showed that the lining of the inner pore consists of hydrophobic amino acids, and the surface of the pore, in the cell's cytoplasm, consists of hydrophilic amino acids.

Intriguingly, the first ten amino acids of the N-terminal peptide tail were also hydrophobic, and the next ten were hydrophilic. Since hydrophobic amino acids attract each other, as do hydrophilic ones, the scientists reasoned that the tail would naturally fit into the pore if it extended deep into the pore's interior.

To test this hypothesis, the scientists altered specific amino acids in the interior of the pore and on the inactivation gate itself. They next looked at how those mutations affected the inactivation gate. These studies revealed that altering the inner pore did, indeed, profoundly affect the inactivation gate.

"Our findings argued that the peptide extended deep into the pore," said MacKinnon. "But we still needed more confirmation. Since the pore is rather

narrow, the question was whether it could open wide enough for a peptide to fit in.”

Thus, the scientists tested whether a relatively large inhibitory molecule, called tetrabutylammonium (TBA), could fit into the pore. X-ray crystallography studies revealed that TBA did fit into the pore -- proving that there was sufficient room for the N-terminal peptide. Furthermore, they showed that mutating the inner pore also affected TBA inhibition of the pore.

“These findings significantly aid understanding of potassium channels in two important ways,” said Aldrich. “They unify the indirect functional work on inactivation over the last fifty years, finally settling the mechanism of this important process. And, they settle a controversy that had arisen over the last couple of years about how an N-terminal peptide could possibly come through one of the four windows on the side of the channel to cause inactivation.”

According to MacKinnon, the findings also explain why drugs that block potassium channels effectively have structural features that resemble the inactivation gate peptide. The structural resemblance can cause serious side effects. “Antibiotics with these features tend to block potassium channels found in the heart and can cause arrhythmias,” MacKinnon said.

The structural studies are likely to improve the design of ion channel blockers, such as those used as anesthetics, said Aldrich. “A large fraction of drugs that act on channels show state-dependent binding -- that is, they may bind better to the inactivated state than to the non-inactivated state,” he said. “So if we know the conformational changes between these different states with different binding affinities, we can design better drugs that will bind to particular states and thereby modulate the channels’ behaviors in certain ways, instead of just blocking them."