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Lipid Molecule Plays Key Role in Neurotransmission

New studies indicate that a specific type of lipid molecule plays a critical role in controlling the behavior of vesicles that store neurotransmitters within neurons. Neurotransmitters are the chemical messengers that neurons release to communicate with one another.

The identification of a regulatory role for the molecule, phosphatidylinositol 4,5 biphosphate, or $\text{PtdIns}(4,5)\text{P}_2$, provides a new view of the operation of the machinery that produces and recycles synaptic vesicles.

Led by Howard Hughes Medical Institute (HHMI) investigator Pietro De Camilli, the researchers published their findings in the September 23, 2004, issue of the journal *Nature*. De Camilli, HHMI investigator Richard Flavell, Reiko Fitzsimonds, and their colleagues at Yale University School of Medicine collaborated on the studies with Timothy A. Ryan and researchers from the Weill Medical College of Cornell University.

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- Pietro De Camilli

Synaptic vesicles are initially loaded with neurotransmitter molecules in the interior of neurons. They then secrete their cargo at synapses—the junctions between neurons. At the synapse, the vesicles undergo a process called exocytosis, in which they fuse with the synaptic plasma membrane and unload their neurotransmitters. Afterward, they are drawn back into the neuron in the process of endocytosis.

According to De Camilli, there had been indirect evidence from cell-free systems, pharmacological and transfection studies that $\text{PtdIns}(4,5)\text{P}_2$ played a role in controlling this process. However, there had not been enough genetic

evidence to establish that this lipid molecule played a regulatory role both in synaptic-vesicle fusion and endocytic recovery.

“We think that this paper provides conclusive support to the hypothesis that PtdIns(4,5)P₂ plays an important role in exocytosis, at least in part via its binding to proteins of the exocytic machinery, and in endocytosis via its binding to the clathrin adaptors,” he said.

In their experiments, De Camilli and his colleagues knocked out the gene encoding an enzyme that produces PtdIns(4,5)P₂ in mice. When they deleted this enzyme, called PIP kinase type 1 gamma, they saw greatly reduced levels of PtdIns(4,5)P₂ in the brain and a deficiency in neurotransmitter secretion. Mice lacking both copies of the gene died shortly after birth.

They then examined synaptic transmission in cultures of neurons from these neonatal mice, and therefore deficient in PtdIns(4,5)P₂, finding a smaller recycling pool of vesicles and a smaller population of vesicles ready for fusion. The researchers then studied the influence of PtdIns(4,5)P₂ on the speed of vesicle recycling after fusion. They used a fluorescent tracer to follow the process by which empty vesicles are internalized and then reused in another round of secretion, finding that the cells lacking PtdIns(4,5)P₂ had a delayed recycling.

To study endocytosis in greater detail, they also transfected cultured neurons with a chimeric protein that is targeted to the synaptic vesicle membrane and that fluoresces brightly when the lumen of the vesicles is exposed to conditions of low acidity, which happens during exocytosis. While held inside the vesicles, where conditions are acidic, the protein exhibits only very dim fluorescence. This experiment allowed the scientists to monitor opening and closing of the vesicles during the processes of exocytosis and endocytosis. The experiments revealed that the neurons lacking PtdIns(4,5)P₂ showed a slowing of endocytosis relative to exocytosis when they were electrically stimulated to trigger neurotransmitter release. Further electron microscopy studies using endocytic tracers revealed that such neurons also showed an impaired clathrin-dependent endocytosis.

According to De Camilli, the studies of the role of PtdIns(4,5)P₂ represent the beginning of exploration into the regulatory role of the class of lipids called phosphoinositides in membrane traffic within neurons. The reversible phosphorylation of their inositol rings makes them powerful regulators of interactions between the membrane bilayer and protein modules present in the cytosol. “This field is in its infancy,” he said. “Although much has been learned about phosphoinositides and membrane traffic in other systems, we still know very little about the regulatory role of phosphoinositides in synaptic physiology. We have begun to explore the role of PtdIns(4,5)P₂, but we would also like to explore the role of other phosphoinositides.”

Basic studies such as those conducted by De Camilli and his colleagues could well lead to insights into diseases that involve abnormal phosphoinositide metabolism. For example, people with Down syndrome have an extra copy of the gene encoding synaptojanin 1, a brain enriched enzyme that degrades $\text{PtdIns}(4,5)\text{P}_2$, and patients with Lowe syndrome, who also have mental retardation, lack another $\text{PI}(4,5)\text{P}_2$ degrading enzyme. Other diseases that can result from abnormal metabolism of phosphoinositides include cancer and diabetes.

“In general, this work helps to emphasize the role of metabolism of membrane lipids in the regulation of membrane processes,” said De Camilli. “Typically, studies of membrane processes focus on the role of proteins, with lipids traditionally thought of as primarily structural components. But more and more it is being appreciated that the chemistry of lipids is important for membrane dynamics, in this case in a specialized area of the synapse,” he said. “Advances in the field of lipids biology may offer new targets for therapeutic intervention in human diseases.”