Neuropeptides, Adenylyl Cyclase, and Memory Storage

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Genetically modified animals are providing us with a new approach to the molecular basis of behavior. In this issue of Science, Feany and Quinn (1) use Drosophila to revisit the idea that neuropeptides have a specific role in memory storage. Their cloning of the Drosophila gene amnesiac reveals that it encodes a novel neuropeptide homologous to mammalian pituitary adenylyl cyclase activating peptide (PACAP).

Peptide messengers have distinctive properties which suggest that they have special functions in the brain (2). Peptides usually are released from nerve terminals during high-frequency neuronal activity as cotransmitters along with a primary small molecule transmitter (such as glutamate or serotonin). Small molecule transmitters act rapidly because they can be quickly degraded and removed from the synaptic cleft. Peptides, in contrast, produce longer-lasting actions, in part because there is no uptake mechanism or degradative system for them in the synaptic cleft. Finally, small molecule transmitters are synthesized and enzymatically packaged in small vesicles at the presynaptic terminal, whereas peptide transmitters are synthesized on ribosomes in the cell body as part of inactive protein precursors, which are then cleaved into smaller, active forms and transported in large vesicles to the nerve terminals.

As cotransmitters, peptides typically enhance the primary transmitter’s action but can also have distinct behavioral effects of their own, modulating homeostasis—particularly thirst (angiotensin) (3), feeding (neuropeptide Y and galanin) (4), and pain (enkephalin) (5). Do peptides also have roles in cognition? Vasopressin and adrenocorticotrophic hormone can enhance memory storage, but this action on memory may be due to secondary effects on arousal, attention, or motivation (6).

With the new results of Feany and Quinn (1), peptides and memory storage have again come to the fore, but now in a genetic context. Feany and Quinn examined amnesiac, a Drosophila mutant that learns normally but forgets rapidly, and found that it encodes a protein precursor related to mammalian PACAP. PACAP, a member of a family of polypeptide hormones that includes secretin, glucagon, and vasoactive intestinal polypeptide (VIP), is expressed in various peripheral tissues and in several regions of the brain, including the hypothalamus and hippocampus (7). PACAP appears to function as a neurotransmitter by interacting with two types of seven-transmembrane-domain receptors. One receptor, which is positively coupled to adenylyl cyclase, also recognizes VIP, the closest family member to PACAP. A second receptor, which recognizes only PACAP, is linked to phospholipase C and adenylyl cyclase (8). In neurons, PACAP increases intracellular cAMP (adenosine 3',5'-monophosphate) and calcium and promotes neurite outgrowth (9).

The predicted amnesiac protein potentially comprises three peptides, two of which are homologous to PACAP. Although the final proof that this PACAP-related gene is indeed amnesiac will require germline rescue experiments, the homology to PACAP suggests that amnesiac increases cAMP by activating adenylyl cyclase. Genetic evidence also supports this idea: In fact, the amnesiac gene was isolated by transposon (P-element) mutagenesis designed to identify mutations that suppress female sterility caused by elevated cAMP levels in dunce mutant flies.

These findings about amnesiac rekindle interest in the role of peptides in memory and further enliven the study of Drosophila learning. These studies date to 1974, when Seymour Benzer and his colleagues, having successfully isolated a variety of important behavioral mutants (in circadian rhythms, courting, visual function, and neural development), turned their genetic screens to the study of learning by testing how flies remember to avoid an odor that has been paired with a shock (10). Using classical biochemical mutagenesis of the X chromosome, Dudai et al. (11) identified dunce, the first Drosophila mutant in learning and memory (11). Additional mutants were then identified quite rapidly, including amnesiac, rutabaga, cabbage, turnip, and radish (12).

Soon thereafter, biochemical experiments found that two of the memory mutants were components of the adenylyl cyclase pathway: dunce encodes a cAMP-dependent phosphodiesterase, and rutabaga encodes a Ca2+-dependent adenylyl cyclase (13). However, the identification of other mutations in these screens proved difficult because behavioral testing is time-consuming and the chemically mutagenized genes were not tagged, making cloning tedious. These obstacles slowed progress in the study of Drosophila learning over the next decade and almost brought it to a halt.

Now, several technical advances have converged to overcome these impediments, moving the study of Drosophila learning to a new level (12, 14). First, Tully et al. greatly improved the behavioral assays and developed techniques for studying both short- and long-term memory (14). Second, Davis (12) carried out enhancer-trap screens for genes expressed in the mushroom body, a portion of the fly’s brain thought to be critical for olfactory learning. Third, Quinn and his colleagues (15) used a heat-shock pro-
enhance the short-term process conditioned stimulus. A peptide module connections in the reflex pathway of the cyclase both short- and long-term facilitation by forms of spatial learning in mammals andenezia, amnesiac analogy, it is tempting to suggest that tide) seems to act in parallel with 5-HT toitory transmitter (small cardioactive pep-citation) stimuli applied to the tail initiate seizure, acting through adenylyl cy-clase-coupled D1/D5 receptors in the pre-frontal cortex of mammals, seems to function in this way to modulate working memory (23). Dopamine similarly modifies LTP in the Schaffer collateral pathway of the rat hippocampus by acting through these receptors (24).

Common to all these learning-related paradigms is the activation of adenylyl cyclase isoforms that are Ca2+-dependent and therefore well suited to associate two stimuli: They can be stimulated by both Ca2+ influx and Gqα (25). Thus, the Ca2+-dependent adenylyl cyclase encoded by the rutabaga gene in Drosophila may be a coincidence detector for classical conditioning in olfactory learning in Drosophila. The amnesia peptides released by the unconditioned stimulus may activate the cyclase through Gqα, whereas Ca2+ influx, produced by activity in the conditioned stimulus pathway, may activate cyclase through Ca2+-calmodulin. That PACAP-mediated transmis-sion at the Drosophila neuromuscular junction is reduced in rutabaga mutant larvae supports this idea (26).

This emphasis on cAMP in certain types of memory storage does not mean that other second messengers cannot have equally important roles in learning. In fact, although cAMP is present in bacteria and may be the most ancient second-messenger system, it typically acts in combination with other second-messenger cascades in eukaryotic cells.

In a broad sense, the role of adenylyl cyclase, PKA, and CREB in memory storage clearly shows that common peptides and second-message-ner cascade can be used for uncommon purposes. To understand how specific memories can be stored in the brain by modulation of kinases that are shared with other cells of the body, we should remember that evolution is a tinkerer—it does not design from scratch. By means of their highly precise connections, the nerve cells of the brain can endow even common molecules with the specific actions necessary to achieve uncommon ends. This is reassuring for the molecular biology of memory storage, as it suggests that even mental functions have not escaped the conservative forces of evo-lution.

References and Notes

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