Alzheimer's Alchemy

Turning an innocuous nerve-cell protein into brain-wrecking beta amyloid

By CAROL EZZELL

The protein bobs along like a buoy adrift on the nerve cell's outer membrane. Suddenly, a whirlpool beneath the surface pulls a patch of the membrane inside the cell, dragging the protein with it. The membrane fuses, closing the surface hole and leaving the protein trapped in a minuscule membrane bubble floating through the cell's watery interior.

Before long, a larger intracellular globule engulfs the tiny bubble, plunging the hapless protein — known as amyloid precursor protein — into an enzymatic hell. Powerful acids from inside the globule attack the protein where it is most vulnerable, chewing it into unrecognizable bits. Its protein dinner digested, the globule — called a lysosome — surfaces through the membrane to spew out the remains, among which is a sticky protein fragment called beta amyloid — the stuff of Alzheimer's disease.

A violent scenario like this may occur countless times each day within the brains of everyone, according to neuroscientists such as Dennis J. Selkoe of Harvard University Medical School in Boston. "All of us have the capacity to make these fragments, and in point of fact, all of us develop beta amyloid deposits with age," asserts Selkoe. "Alzheimer's is just a quantitative exaggeration of something that happens normally."

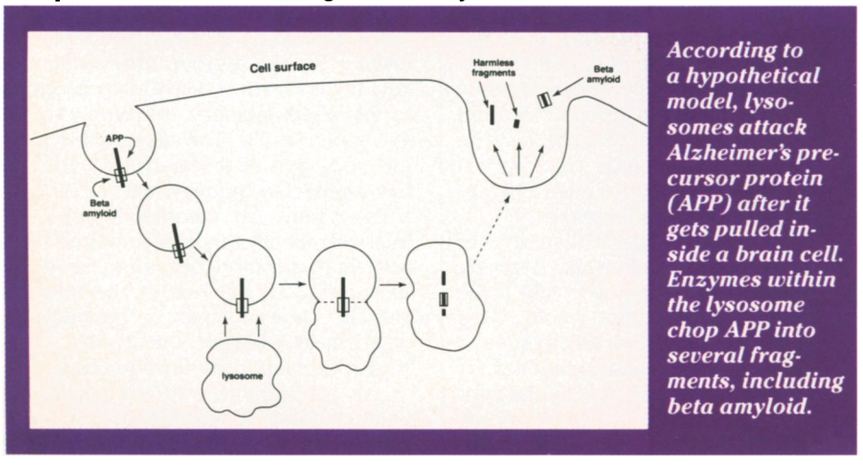
This theory — shared by a growing contingent of Selkoe's colleagues in Alzheimer's research and supported by recent studies — is causing a minor revolution among scientists studying the disease. Neuroscientists are now exploring all phases of beta amyloid production in nerve cells, looking for clues as to why some people make more of the destructive protein than others and go on to develop Alzheimer's disease. They are also turning up molecular evidence of how beta amyloid achieves its effects, laying waste to the memories and personality of an otherwise healthy individual.

Eighty-five years after its first description as a distinct malady by German neurologist Alois Alzheimer, Alzheimer's disease has become so commonplace that people joke about having it when they can't find their car keys. The National Institute on Aging estimates that Alzheimer's currently afflicts roughly 4 million Americans and projects that the disease will eventually strike half of all people who live beyond the age of 85. Although patients in the initial stages of Alzheimer's may experience only short-term memory lapses and occasional confusion, as the disease progresses they often revert to a childlike mentality and must be waited on and watched around the clock by family members or placed in a nursing home. Eventually, most Alzheimer's patients lose control of their bodily functions, and devolve into a vegetative state. Finally, some die of pneumonia, while others die in their beds from no apparent cause.

Doctors can only diagnose Alzheimer's through a process of elimination, ruling out other disorders such as a slight stroke, a brain tumor, or even an adverse drug reaction. A definitive diagnosis must await death and an autopsy, when a pathologist can view the telltale "senile plaques" that pock the brains of Alzheimer's victims.

In 1984, researchers discovered that these senile plaques consist of a central core of beta amyloid protein, surrounded by a cluster of abnormal nerve cells clogged with twisted fibers called neurofibrillary tangles. Last year, a research team led by John Hardy of St. Mary's Hospital Medical School in London found that some people with an inherited, early-onset form of Alzheimer's shared a specific mutation in their gene for amyloid precursor protein (APP) (SN: 2/23/91, p.117). Researchers have so far not determined the function of APP, which is embedded in the outer cell membrane.

Following the publication of Hardy's results — and several successive papers by other groups reporting similar findings — scores of Alzheimer's researchers began scrutinizing how APP gets converted into the fateful beta amyloid. Many became convinced that beta amyloid is an abnormal protein produced only when some biochemical process in the brain goes awry, possibly triggered by a mutation in APP. By understanding this abnormal process, these researchers reasoned, they might find a way to block beta amyloid production and treat or prevent Alzheimer's.



According to a hypothetical model, lysosomes attack Alzheimer's precursor protein (APP) after it gets pulled inside a brain cell. Enzymes within the lysosome chop APP into several fragments, including beta amyloid.

Back-to-back reports in the Feb. 7 SCIENCE challenge this notion, however. Two teams of researchers led by Steven G. Younkin of Case Western Reserve University in Cleveland describe studies showing that there are no "normal" or "abnormal" APP-degrading processes. They report that both normal and Alzheimer's nerve cells possess two separate pathways for breaking down APP — the secretory pathway, which yields apparently harmless protein fragments, and the lysosomal pathway, which carves beta amyloid from APP.

In the first study, Younkin and a group of colleagues chopped up cells taken from the brains of individuals who died of Alzheimer's and persons who died of other causes. They poured slurries of each through separate tubes filled with microscopic beads coated with special antibodies made to stick specifically to APP. The antibody-coated beads attracted the APP in the slurries but allowed all other proteins to pass through the tubes. The researchers then recovered the APP by pouring a solvent through the tubes to free the APP from the tiny beads.

When they separated the two purified APP samples on a gel according to size, they found that both contained intact

APP. But to their surprise, they discovered that both samples also contained five shorter APP fragments, the two largest of which contained the beta amyloid protein.

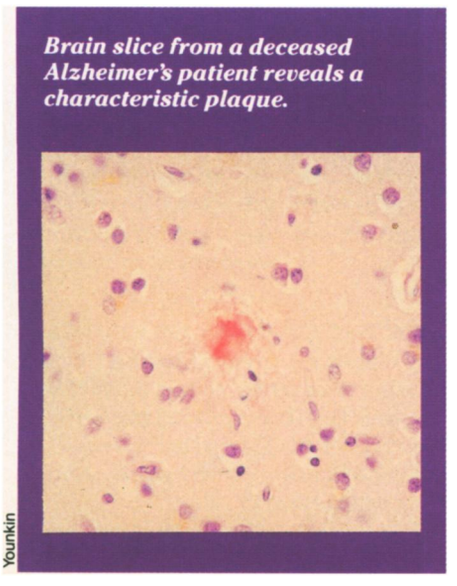
"This shows that amyloid isn't just produced in Alzheimer's brains," concludes Younkin.

In the second SCIENCE paper, Younkin and another group — including Harvard's Selkoe — demonstrate that APP fragments with the potential to yield beta amyloid are formed within lysosomes, the scavengers of the cell. These globules of acids and enzymes usually roam the cell's interior, digesting worn-out proteins and other cellular refuse. Younkin and his colleagues treated normal nerve cells with chemicals known to block lysosomal enzymes. When they chopped and filtered the cells, they found two kinds of large APP fragments, neither of which contained the full beta amyloid protein.

Earlier research by others had shown that these two large fragments are the products of a single biochemical reaction involving an enzyme called "secretase," which clips off APP just where it pokes through the cell membrane, much like shaving off a hair. Because most of the beta amyloid portion of APP protrudes through the membrane, this process destroys beta amyloid.

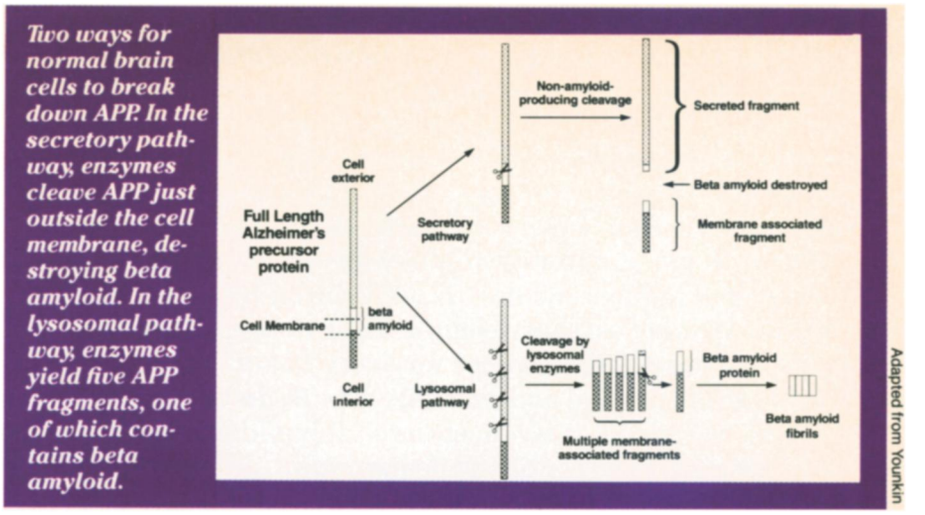
Younkin asserts that both the lysosomal and the secretase pathways occur normally in healthy brains. Alzheimer's disease results, he says, when something tips the biochemical balance within nerve cells, favoring the production of small, beta amyloid-containing APP fragments over larger, harmless APP fragments.

"We're really getting very close to identifying the pathway that produces amyloid," says Younkin. "Once we've identified that pathway, then we can figure out all of the things that modify it in a way that either enhances or undermines amyloid deposition. Once we get our hands on the processing pathway and the mechanisms involved, we'll then be in a position to begin to look for drugs that block it."



Brain slice from a deceased Alzheimer's patient reveals a characteristic plaque.

Younkin



Two ways for normal brain cells to break down APP. In the secretory pathway, enzymes cleave APP just outside the cell membrane, destroying beta amyloid. In the lysosomal pathway, enzymes yield five APP fragments, one of which contains beta amyloid.

Adapted from Younkin

But what steers more APP into the lysosomal pathway in some people, yielding more beta amyloid? Younkin and Samuel Gandy of Rockefeller University in New York City think the answer might rest with a ubiquitous cell enzyme called protein kinase C (PKC).

PKC — which plays a significant role in the biochemistry of learning and memory (SN: 5/25/91, p.328), among a variety of other functions — acts by tacking phosphate molecules onto specific sites on other molecules. In a study recently submitted for publication, Younkin and Gandy report evidence that the secretase pathway requires PKC in order to break down APP into harmless fragments. Younkin proposes that a slight reduction in PKC could tip the balance away from the secretase pathway, and toward the beta amyloid-generating lysosomal pathway. This reduction, he theorizes, might have a variety of causes, possibly including a mutation in the gene that directs the production of PKC.

On the other hand, neuroscientist Richard J. Wurtman of the Massachusetts Institute of Technology in Cambridge has another theory. He and colleagues at Harvard University Medical School and Boston University School of Medicine believe a membrane phenomenon they call "autocannibalism" may be to blame for steering cells onto the lysosomal pathway that leads to beta amyloid.

In the March 1 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Wurtman's team reports evidence that nerve cells in the brains of people who develop Alzheimer's have lower levels of key membrane molecules called phospholipids than do nerve cells in healthy individuals. One of these phospholipids, phosphatidylcholine, is also a precursor of acetylcholine, a chemical messenger between nerve cells that is reduced in the brains of Alzheimer's patients. The researchers posit that nerve cells hungry for choline compounds raid their own cell membranes for phosphatidylcholine; this leaves holes on the underside of the membrane and possibly exposes portions of APP to marauding lysosomes.

Wurtman's team is now conducting experiments seeking to determine whether such membrane defects do increase production of beta amyloid. "These are quite interesting neurochemical facts," says Wurtman. "If they can be tied together, so much the better."

To complete the chain of events that leads to Alzheimer's, a team led by Mark P. Mattson of the University of Kentucky in Lexington has discovered how beta amyloid kills nerve cells. In the February JOURNAL OF NEUROSCIENCE, researchers working with Mattson and at Athena Neurosciences Inc. in South San Francisco, Calif., report that beta amyloid disrupts the ability of nerve cells to regulate their internal calcium levels.

Mattson's group measured calcium levels inside nerve cells grown in the laboratory after exposing them to beta amyloid. The researchers found that although beta amyloid itself produced no toxic effects, it increased the calcium influx mediated by glutamate. This amino acid normally excites nerve cells, causing them to take in calcium, but it can sometimes kill them by causing them to draw in excessive calcium amounts. Therefore, Mattson's team concluded, beta amyloid indirectly kills nerve cells by flooding them with calcium.

Fueled by developments like Mattson's, Wurtman's and Younkin's, Alzheimer's researchers will continue to focus their inquiries on beta amyloid for some time to come. "In the last five years, we've gone from 'maybe amyloid might be doing something' to pretty strong evidence that amyloid is the culprit in Alzheimer's," says Younkin. "And we're going to keep on going until we figure out how to stop it." □