

MAY 18, 2000

Alzheimer's - Triggering Enzyme Identified

Researchers have identified an enzyme that responds to toxic insults to brain cells and triggers neurodegeneration akin to that seen in people with Alzheimer's disease.

The discovery, reported in the May 18, 2000, issue of the journal *Nature*, proposes a common mechanistic link between the brain cell injury associated with Alzheimer's disease and known causes of neurotoxic damage, including oxidative stress, excitotoxic chemicals and oxygen starvation.

"While other neuronal insults that disrupt calcium homeostasis were believed to predispose people to neurodegeneration, there has not been a good molecular mechanism to explain such damage. I believe that calpain provides at least part of the answer."

— Li-Huei Tsai

Writing in *Nature*, Howard Hughes Medical Institute investigator Li-Huei Tsai and colleagues at Harvard Medical School suggest that the enzyme calpain could be a target for drugs designed to slow or stop the progression of brain damage caused by Alzheimer's disease. Tsai's team found that calpain slices apart a regulatory protein called p35 that aids in the development of neural tissue. Calpain splits p35 into two proteins, p10 and p25. The presence of p25 in brain cells triggers formation of some of the deadly snarls of protein that can damage or kill those cells.

Last December, Tsai and her colleagues at Harvard Medical School showed that the p25 protein fragment maintains the activity of p35 and can switch on cdk5an enzyme normally turned on by p35 that catalyzes the construction and maintenance of neural tissue during development.

Tsai's group showed that trouble starts when p25 loses a critical targeting segment that is contained in p35, so p25 "turns on" cdk5 and allows it to wander through the cell's cytoplasm hyperphosphorylating other

proteins notably, a cytoskeletal building-block protein called tau. The altered tau protein becomes less able to attach to cytoskeletal proteins and aggregates into the lethal neurofibrillary tangles seen in brain cells ravaged by Alzheimer's disease.

After finding that p35 is cut into two proteins, Tsai's team's next quest was to identify the enzyme responsible for cleaving p35. A key to the enzyme's identity emerged when Tsai and her colleagues found that increased levels of calcium played a role in p35 cleavage.

"In cultures of mouse brain cells, we observed that a drug that increases intracellular calcium also induces p35 cleavage in neurons," she said. "Next, using an *in vitro* system, we found that adding calcium to fresh brain lysates also induced cleavage of p35."

The scientists then treated the brain lysates with several inhibitors of calcium-dependent enzymes to see whether a single inhibitor would block p35 cleavage. They found that inhibitors of calpain completely blocked p35 cleavage. Further experiments verified that calpain is the key enzyme that slices p35.

The link between neurotoxicity and calpain-induced p35 cleavage surfaced when the scientists subjected cultured neurons to neurotoxic damage while blocking calcium.

"We found that when we blocked calcium influx from outside the cell or prevented calcium release from inside the cell, we prevented p35 cleavage," said Tsai. "This observation strongly indicated that these different neurotoxic processes disrupt calcium levels, and therefore lead to calpain activation."

In another important finding, Tsai and her colleagues showed in cell culture studies that p35 cleavage is induced by A β peptides, small proteins that form the brain-cell-clogging plaques in Alzheimer's disease. The formation of such plaques in brain cells is considered one of the hallmarks of Alzheimer's disease and is believed to contribute to the death of brain cells. The scientists also showed that inhibiting either cdk5 or calpain considerably reduced A β peptide-induced cell death.

"These observations are very exciting because until now only A β peptides have been implicated in the pathogenesis of Alzheimer's disease," said Tsai. "While other neuronal insults that disrupt calcium homeostasis were believed to predispose people to neurodegeneration, there has not been a good molecular mechanism to explain such damage. I believe that calpain provides at least part of the answer."

According to Tsai, drugs that inhibit either calpain or cdk5 might prove useful in reducing neurodegeneration related to Alzheimer's disease.

"Complete inhibition of calpain or cdk5 might not be desirable," she added, "because cdk5 is necessary for normal neuronal functioning, and calpain may also be necessary."

"Thus, for therapeutic purposes, it might be better to use drugs that only inhibit some enzymatic activity, with the goal being to delay the onset of the disease," said Tsai.

She also speculated that diagnostic tests to detect the presence of p25 in cerebrospinal fluid might provide an early indicator of the presence of Alzheimer's disease, and enable physicians to begin treatment earlier.

Tsai emphasized that although calpain seems to play a central role in the cellular damage associated with Alzheimer's disease, "Alzheimer's is a multifactorial disease, and this probably is just one of the mechanisms."