Blocking destruction of defective proteins unexpectedly delays neurodegeneration in mice

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One might expect that ridding a brain cell of damaged proteins would be a universally good thing, and that impairing the cell's ability to do this would allow the faulty proteins to accumulate within the cell, possibly to toxic levels. So a lot of scientific effort has gone into looking for ways to enhance the process by which cells dispose of banged-up proteins.

But this thinking may need some revision, according to a new study from the Stanford University School of Medicine. Senior author Thomas Sudhof, MD, professor of molecular and cellular physiology, and his fellow researchers have unexpectedly found that inhibiting the process by which damaged proteins are ordinarily broken down within cells both delayed the onset of symptoms in laboratory mice that are highly prone to neurodegeneration and significantly increased their longevity.

The study, to be published online Aug. 15 in *Science Translational Medicine,* also showed that blocking the activity of cells' in-house garbage disposals — known in the biology business as proteasomes — in nerve cells taken from the neurodegeneration-prone mice restored several key biochemical characteristics necessary for the cells' healthy function.

Although Sudhof cautions that more research is needed, the findings pose a challenge to prevailing beliefs about the pathology of certain neurodegenerative disorders.

"The current consensus in neuroscience favors a therapeutic strategy of trying to accelerate, rather than impede, the disposal of damaged proteins that accumulate in the brains of patients with Alzheimer's, Parkinson's, Huntington's and other neurodegenerative diseases," said Sudhof, who is also the Avram Goldstein Professor in the School of Medicine.

Proteasomes are cell components that destroy damaged proteins. Not just nerve cells but virtually all cells in creatures ranging from yeast to humans contain multitudes of these tiny tube-shaped machines, which suck the defective proteins into their holes and chop them into smithereens.

Examining brain tissue from deceased Alzheimer's and Parkinson's patients, Sudhof's team observed that an aspect of the degenerative process identical to the one they had prevented in lab mice was occurring, suggesting that their findings in the animal model might prove relevant to these and other human diseases as well.

The particular defective protein whose demolition was staved off in Sudhof's study, called SNAP-25, plays a key role in the release of chemical signals that nerve cells use to communicate with one another. But like all proteins, SNAP-25 can't do its job until it is slapped into shape.

"Structure equals function" is a watchword of biochemistry. Proteins — the molecular creatures that do the bulk of the work in every living cell — are initially produced as long linear sequences of small chemical subunits that progressively get strung together like beads on a string. But the string is just a string until it assumes a specific structure, typically with help from one or more "chaperone" molecules that midwife it into its correct conformation.

Like a mail carrier's feet, overworked proteins can eventually go flat. Misfolded and therefore no longer functional proteins may, alternatively, be quickly reconditioned and put back on the job or get chemically "barcoded" for demolition at the hands of proteasomes.

SNAP-25 is used by many types of cells but works particularly hard in nerve cells, or neurons. To transmit signals to one another, neurons release specialized chemicals into small gaps called synapses that separate one neuron from the next in a relay. Prior to release, those chemicals are sequestered within membrane-bound packets, or vesicles, inside the neuron. Every time one neuron transmits a signal to the next — which can be more than 100 times a second — hundreds of tiny chemical-packed vesicles approach the edge of the first neuron and fuse with its outer membrane. A fused vesicle's inner surface becomes part of the neuron's outer surface (just as would happen if a small bubble merged with a larger one surrounding it), and its stored contents spill out into the synapse.

To make this happen, vesicles must be actively coerced into close contact with neurons' membranes. This is accomplished by a cluster of proteins that serves as the molecular equivalent of a clamp. In the course of repeated bouts of neuronal firing, SNAP-25 — a key constituent of this clamping complex — can get bent out of shape, rendering the entire clamp assembly useless. The more a neuron fires, the more molecules of SNAP-25 get deformed over time.

Some years ago, Sudhof's group used a sophisticated genetics technique to create a strain of laboratory mice lacking a chaperone molecule that assists in the proper refolding of SNAP-25. These mice seem quite normal early in life. But the gradual failure of the clamping action necessary for vesicle fusion and neuronal signaling causes the neurons to die off, eventually triggering behavioral symptoms and early death. Sudhof has used these mice extensively as a model system for

understanding misfolded proteins and degenerative disease.

In the new study, giving the chaperone-deficient mice injections of either of two proteasome-inhibiting drugs (lactacystin and epoxomicin) once every five days — probably far from an optimal regimen, but at least a feasible animal experiment, Sudhof said — delayed typical outward symptoms of their neurodegenerative disorder by as much as 30 percent and prolonged their survival by close to 20 percent. Next, the researchers incubated neurons from the experimental mice in solutions with or without proteasome-inhibiting drugs. Biochemical measurements showed that, while overall stores of SNAP-25 in the neurons incubated with or without drugs were the same, the activity of the clamping complex was restored to normal levels in the drug-soaked neurons. (In the drug-free neurons, clamp activity remained hugely impaired.)

Clearly, preventing the breakdown of misfolded SNAP-25 molecules increased their levels in neurons — an outcome that one would intuitively think would be toxic, Sudhof said. But the experiments in the new study instead suggest that misfolded proteins, either randomly just from being bounced around in the surrounding environment or with the active assistance of "generic" chaperone molecules, can revert to normal shapes and jump back into the fray. As faulty SNAP-25 accumulates in the cell, more of it gets repaired, with beneficial downstream implications for molecular-clamp activity, vesicle-membrane fusion and better chemical signaling by neurons. Because neurons have to signal effectively in order to survive, fewer of them die.

A number of neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases are associated with the occurrence of damaged proteins. It is a widely held view among investigators in the field that the buildup of these damaged proteins substantially contributes to the disease process.

Sudhof cautioned that, while it is possible that proteasome-inhibiting drugs could, paradoxically, turn out to slow the progress of these and other human degenerative conditions, this was far from proven. However, far more powerful proteasome-inhibiting drugs than the old, off-patent drugs Sudhof and his teammates gave their experimental mice are already in clinical use for certain cancer indications, so they could rapidly be deployed in preclinical studies testing this possibility.

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