Stroke-Damaged Neurons May Commit Cellular Suicide

NEWS

Recent evidence that neurons die from apoptosis in oxygen-deprived brains and also in the brains of Alzheimer's patients suggests new approaches to therapy

When human beings commit suicide, it's almost always a tragedy. But within the organism, the cellular suicide that goes by the name of apoptosis is vital to life. It prunes tissues during embryonic development and removes damaged cells in the adult in a neat, orderly way. But apoptosis has a dark side as well: If it's turned on at the wrong time, crucial cells may die off. Over the past few years, researchers have come to suspect that's just what happens when a stroke or heart failure deprives the brain of oxygen.

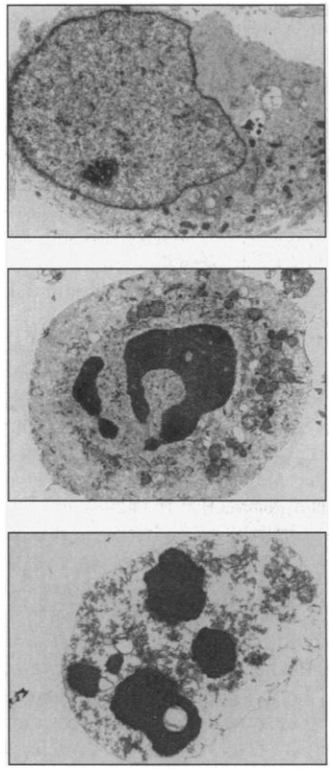
When the blood supply to part of the brain is blocked, as in a stroke, neurons in the most severely affected area die immediately from oxygen starvation, known as ischemia. But a long-standing puzzle in neurology is what causes the more gradual loss of neurons in the region outside the stroke's core, where the oxygen supply is reduced but not eliminated. Recent experiments in rats and mice suggest a possible explanation: Some cells that might otherwise recover from the ischemia may be dying because the injury triggers their suicide programs. When researchers induced brain ischemia in lab rodents by temporarily cutting off blood flow to the animals' brains, for example, they found dying neurons there that show some key criteria of apoptosis. In particular, the cell death seemed to be controlled by caspases, protein-clipping enzymes that orchestrate the cell's death program (*Science*, 3 April, p. 32).

These findings haven't proved that the dying neurons are truly undergoing apoptosis, because the cells don't completely fit the textbook description. Because of all the stereotyped criteria it is expected to meet, "apoptosis seems to be a charged word," says neuroscientist Michael Moskowitz of Harvard Medical School in Boston. "Maybe it's better to just talk about 'caspase-mediated cell death.'" But whatever it's called, he says, the cell death should be a good target for therapeutic drugs aimed at limiting stroke damage.

Indeed, recent work already suggests that caspase inhibitors should be added to the list of potential new drugs for stroke. What's more, research on a related problem—brain damage caused by oxygen starvation during or just after birth—hints that the same drugs may be able to limit that type of damage as well.

Until about 5 years ago, most researchers thought that neurons killed during strokes die not by an orderly program of apoptosis but simply by breaking apart in an uncontrolled form of death called necrosis. That's a messy way to go—necrotic cells spill their contents, which can attract potentially harmful inflammatory cells to the damaged site. But in the mid-1990s, several research groups questioned that dogma and produced evidence suggesting that at least some of the brain neurons damaged by stroke succumb instead to apoptosis. This is a much neater way of dying in which the cell disassembles its DNA and breaks up its contents into membrane-wrapped packets, which can be cleared away without causing inflammation.

The first clue came when researchers showed that the protein-synthesis inhibitor cycloheximide reduces brain damage in laboratory rats given experimental strokes. They chose cycloheximide for the experiments because it was known to inhibit apoptosis. But as neuroscientist and stroke researcher Dennis Choi of Washington University in St. Louis notes, "that early work can be criticized because [cycloheximide] is relatively nonspecific." Because the drug blocks protein synthesis generally, researchers couldn't be sure which of its many effects was behind the stroke results.



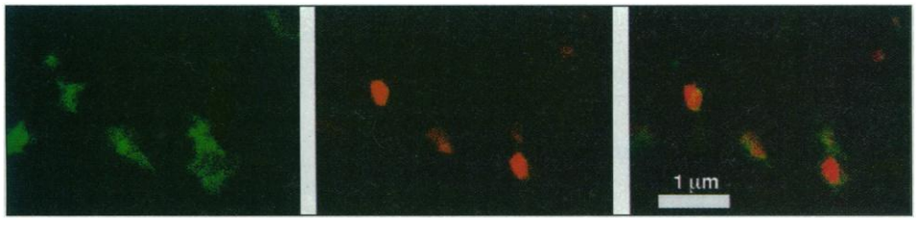
CARL COTMAN, JOHN WATT, AND CHRISTIAN PIKE

Death throes. Compared to a normal neuron *(top)*, two neurons undergoing apoptosis show a dark, dense nucleus *(middle)* and a nucleus breaking up *(bottom)*, although the neurons are still held together by a membrane.

But around the same time, several groups studying rat brain neurons following strokes found other signs of apoptosis: DNA breaks and an overall granular appearance caused by the condensation of the chromosomes in the decomposing nuclei. They concluded that the neurons were dying by apoptosis.

Those findings soon became controversial. Neuroscientist Alastair Buchan of the University of Calgary, one of the researchers who found the DNA breaks, had second thoughts about whether the brain neurons in which they occur are truly undergoing apoptosis. Under closer examination with electron microscopy, he says, the contents of the dying cells don't appear to be neatly packaged in membranes, as they are in apoptotic cells. Instead, "the membranes are completely shot."

In addition, a collaborator of Buchan's, John MacManus of the National Research Council of Canada's Ottawa lab, who has done more extensive tests on the DNA in the dying neurons, found that it is cut up differently than the DNA in true apoptosis. Molecular tests showed that instead of being blunt, the ends of the DNA are ragged,



SHOBU NOMURA AND MICHAEL MOSKOWITZ

Apoptosis tracks. As indicated by the right-hand panel, two signs of apoptosis, caspase-3 production (green stain) and cutup DNA (orange stain), coexist in these neurons from ischemic mouse brains.

suggesting that it is being chopped not by the enzyme that normally cuts the DNA during apoptosis but by some other DNA-degrading enzyme.

MacManus and Buchan argue that the neuron death in stroke is something between apoptosis and necrosis. They see this not as a setback but as an opportunity. "There may be processes in effect here ... that are specific to ischemic neurons," says MacManus. And that, Buchan adds, could "yield us a lot of therapeutic targets, which we badly need."

Others maintain that neurons dying by apoptosis shouldn't be expected to look exactly like other apoptotic cells. Nancy Rothwell of the University of Manchester in the United Kingdom notes that apoptosis was first described in dividing immune cells. "I would be surprised if the criteria established in one type of cell are going to look identical in another," she says, especially in neurons, which do not divide.

The view that apoptosis is the cause of death for some of the neurons killed by stroke got a big boost last year when Michael Moskowitz, Junying Yuan, and their collaborators at Harvard Medical School reported evidence suggesting that caspase activity plays a role in cell death after stroke. The team induced strokes in mice whose caspases had been blocked in either of two ways: by using peptides that inhibit a range of the enzymes, or by a genetic engineering trick that specifically inhibits a particular caspase called caspase-1.

Compared to control animals, caspase inhibition by either method decreased the area of stroke damage by up to 40% or 50%, says Moskowitz. What's more, the neurons were not only saved from dying but seemed to remain in working order; the animals had fewer movement and sensory impairments than controls.

Although many researchers consider caspase activation to be an even more reliable hallmark of apoptosis than the DNA changes measured in the earlier experiments, these results don't settle the issue. Caspase-1, the caspase knocked out in the genetically altered mice and one of those targeted by the peptide inhibitors used, lives a double life. In addition to playing a direct role in apoptosis, it also cleaves and activates interleukin-1β (IL-1β), a signaling molecule of the immune system that triggers inflammation. And inflammation after strokes also causes neuron damage and death.

Rothwell's team at the University of Manchester has shown that IL-1β activity goes up in the brains of rats after stroke and seems to contribute to the neuron damage that ensues. When they block IL-1 action with a protein called IL-1 receptor antagonist (IL-1ra), Rothwell reports, "it reduces ischemic brain damage by greater than 50%." Indeed, she adds, "there is considerable commercial interest" in developing IL-1ra into a possible stroke therapy.

But not all the effects of caspase inhibitors can be explained away by inhibition of inflammation. Moskowitz says his group detected the neuron protection before the time when inflammatory changes typically occur in the brain. What's more, other evidence suggests that caspase-3, which has no direct role in inflammation but is key to apoptosis, also helps kill neurons after ischemia. In May, Moskowitz, Yuan, and their colleagues reported that they had used antibodies that specifically recognize activated caspase-3 to show that caspase-3 activity increases in rodent's brains after stroke. Also in the past few months, Roger Simon and his colleagues at the University of Pittsburgh School of Medicine and a group at Eli Lilly and Co. in Indianapolis reported that caspase-3 gene expression is up as well in rats with global ischemia, the type of brain oxygen starvation that occurs during heart failure. "This puts the apoptosis story on a firmer footing," says Moskowitz.

Researchers will now want to follow up on this work by exploring the safety of known caspase inhibitors for use in stroke therapy, and by searching for even better drugs. And this may not be the only type of ischemia in which they might prove helpful.

Most neuroscientists agree that neurons in the brains of newborn rats deprived of oxygen undergo a death that has all the features of textbook apoptosis, including the intact membranes missing in adult brains. This led neurologist David Holtzman of Washington University to wonder whether caspase inhibitors might prevent cerebral palsy in human babies that have suffered ischemia during difficult births and those born weighing less than 1500 grams, half of whom develop some form of ischemic brain damage due to their immature lungs and brains. If neural apoptosis is to blame, says Holtzman, "one would predict that if you inhibited caspases ... you would protect the brain."

Animal tests of his hunch have looked promising. Holtzman's team reported earlier this year that apoptosis inhibitors given to newborn rats within 3 hours after the researchers had induced brain ischemia protected brain neurons from death. They are now studying rats treated this way to see if the neurons they save go on to function normally. At least one pharmaceutical company is interested in trying this approach to treat infants at risk for cerebral palsy, Holtzman says. So in young as well as old brains, protecting neurons from untimely death may provide a new lease on life. **–MARCIA BARINAGA**

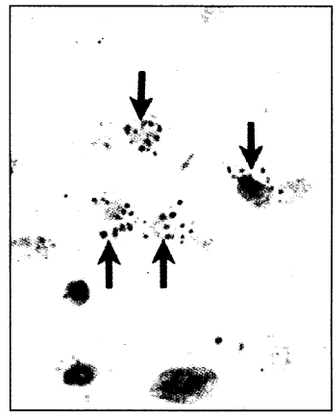
Is Apoptosis Key in Alzheimer's Disease?

NEWS

Cell suicide may play a role in Alzheimer's disease, although the value of blocking it is not yet known

Researchers studying diseases as devastating as Alzheimer's want to explore every lead that could produce a treatment or cure. So while they have several good potential culprits in the nerve cell death that characterizes the disease—most notably a toxic protein called β amyloid (Aβ)—neuroscientists are also following up on evidence that some of the dying neurons commit a form of suicide called apoptosis. What remains to be seen is whether the cellular equivalent of suicide intervention will help slow the disease's progress.

Researchers have found that the brains of Alzheimer's patients contain dying neurons that display certain characteristic signs of apoptosis, such as DNA breaks. Even more intriguing, three proteins already linked to Alzheimer's pathology—Aβ itself, along with two others called presenilins—seem to drive cells into apoptosis when conditions are right. Because of the lack of good animal models of the disease, no one has been able to test whether inhibitors of apoptosis can protect against cell death in Alzheimer's, as they can in animal models of stroke (see p. 1302).



CARL COTMAN, JOSEPH SU, AND CHRISTIAN PIKE

Telltale dots. Neurons in a brain from an Alzheimer's patient show a dotlike staining pattern characteristic of broken DNA in cells undergoing apoptosis.

But if apoptosis does turn out to play a role in the nerve cell loss, the finding could lead to badly needed new Alzheimer's therapies—a goal that keeps researchers interested despite the uncertainties. "Will [preventing apoptosis] wind up being a good thing?" asks Steven Younkin of the Mayo Clinic in Jacksonville, Florida. "That is an open question ... but I don't think that means you shouldn't pursue [it]."

The notion that apoptosis occurs in Alzheimer's came to light in 1993 with the work of two research teams, Carl Cotman's at the University of California, Irvine, and Gianluigi Forloni's at the Institute of Pharmacological Research in Milan, Italy. The teams showed that Aβ, which builds up in the brains of people with Alzheimer's, caus-