Chilled Brains

Hibernating animals may hold clues to novel stroke treatments

By JOHN TRAVIS

As the last leaves tumble slowly to the ground and snow begins to blanket much of the country, many animals have prepared for the winter's scarcity of food by falling into the long slumber called hibernation. Secure in their burrows, some of the animals undergo remarkable physiological changes, including dramatic reductions of body temperature and heart rate.

Take the extraordinary case of a hibernating arctic ground squirrel. Its heart beats only a few times a minute, and its body temperature drops below the freezing point of water.

"It's hard to detect any kind of heartbeat in them. It's really difficult to tell if they're dead or alive. They're just cold little balls," says Kelly L. Drew of the Institute of Arctic Biology at the University of Alaska Fairbanks.

How hibernating creatures induce and survive this near-death state, known as torpor, has long fascinated scientists studying animal physiology. More recently, biomedical investigators have also begun to take an interest.

In particular, two research groups hoping to unearth novel ways of treating stroke have started to examine how the brains of squirrels endure the rigors of hibernation. They described some of their initial results at the Society for Neuroscience meeting in New Orleans in October.

The dearth of effective treatments for stroke is the motivation for this unusual research effort, explains John M. Hallenbeck, chief of the stroke branch at the National Institute of Neurological Disorders and Stroke (NINDS) in Bethesda, Md.

Sometimes called "brain attacks" to highlight their similarity to heart attacks, most strokes result when something, say a clot or a ruptured blood vessel, interrupts the flow of blood to the brain, creating a condition called ischemia. The only proven stroke therapy is prompt use of tissue plasminogen activator, a clot-busting agent, and even that may not help the majority of stroke patients.

Strokes produce waves of brain cell destruction. The halting of the blood flow stems the brain's supply of oxygen and glucose, immediately slaying a core group of cells. Even after blood flow resumes, additional groups of nearby cells continue to succumb to the stressful event. "This is viewed as brain tissue that is potentially salvageable—if you knew what to do," says Hallenbeck.

Several years ago, Hallenbeck and Kai U. Frerichs of Brigham and Women's Hospital in Boston wondered what happens to blood flow to the brain when squirrels hibernate. The amount of blood reaching the brain plummets by 90 percent or more, the scientists discovered.

"They have a very low blood flow, almost a trickle, through their brains, which they tolerate for a long time," marvels Hallenbeck.

Consequently, hibernating brains face limited blood-borne supplies of glucose and oxygen, the primary molecules that cells use to generate energy. Unlike brains that have undergone a stroke, however, hibernating brains suffer no ill effects.

These contrasting outcomes highlight the primary difference between hibernation and stroke. "Hypoxia [insufficient oxygen] is the number-one consequence of a stroke. It's not in hibernation," notes Larry C.H. Wang, a hibernation researcher at the University of Alberta in Edmonton.

Hibernating animals reduce the biochemical activity of cells, including brain cells, to such low levels that even a dramatic reduction in blood flow does not create a shortage of oxygen, explains Wang.

As part of this shutdown, protein production is severely restricted in the brains of hibernating animals, scientists have recently discovered. "Biosynthesis of proteins is virtually arrested for weeks," says Frerichs.

Curious as to whether this phenomenon stems merely from the brain cells' being colder than normal, Frerichs and his colleagues removed slices of tissue from the hippocampal region of hibernating squirrels' brains. Kept alive in test tubes, the tissue continued to exhibit suppressed protein synthesis, even when warmed to 37°C. Yet when ribosomes, the protein-making factories in cells, were isolated from the hippocampal tissue, they created proteins at a normal rate.

Something inside the brain cells of a hibernating animal may actively suppress the creation of proteins, perhaps to conserve the limited energy available, suggests Frerichs. Or, he adds, cells may simply divert the energy normally devoted to protein assembly to more immediate needs, such as maintaining appropriate concentrations of ions within the cells. If ions aren't properly balanced, cells will swell and die.

The researchers are now looking for the molecular mechanisms by which a hibernating animal lowers its brain's need for oxygen and glucose. With such knowledge, propose Frerichs and Hallenbeck, physicians may someday be able to prevent cell death from stroke, and perhaps from other head injuries, by inducing a hibernationlike state in the human brain.

"It's conceivable that if you knew how hibernators switch themselves off without dissolving their brains, that might be a tool to prevent ischemic damage in other species," says Frerichs.

The second research team exploring the relevance of hibernation to stroke took shape last year, when Margaret E. Rice of New York University Medical Center visited a colleague in Fairbanks. During the trip, Rice met Drew, who had been studying the brain chemistry of hibernating squirrels.

Rice's research has centered on how animals use antioxidants, molecules that defuse free radicals, the destructive molecules generated when cells metabolize oxygen to produce energy. One such defender is ascorbic acid, better known as vitamin C. While people and all nonhuman primates must obtain ascorbic acid from their food, most other animals synthesize the vitamin in their liver.

In earlier work, Rice had examined the amount of ascorbic acid in the brains of turtles. These animals can accumulate a striking amount of vitamin C—five times that observed in the human brain. Rice suspects that this antioxidant bounty protects turtles when they surface after long periods underwater and ravenously consume oxygen to supply their brains and bodies with energy.

Merging their interests, Rice and Drew decided to investigate antioxidant concentrations in hibernating arctic ground squirrels. They've now found that the amount of ascorbic acid in the blood of

Frerichs

A hibernating ground squirrel curled up in its laboratory burrow.

hibernating animals rockets to four times that measured during nonhibernating periods. Moreover, the amount of ascorbic acid in the cerebrospinal fluid that bathes the central nervous system doubles during hibernation. "It seems an important strategy to build up this extracellular store of ascorbic acid," says Rice.

The researchers also noticed that when a squirrel roused temporarily from its torpor, a periodic occurrence for most hibernators, its vitamin C supply returned to normal within hours.

"When they go down into hibernation, ascorbic acid goes up. And as soon as they warm up, it goes away really fast," says Drew.

The investigators believe that the increased supply of vitamin C protects the squirrel's brain from the rush of free radicals that occurs when torpor ends, blood flow to the brain resumes, and cells begin vigorously generating energy. To confirm this hypothesis, the scientists plan to reduce the amount of ascorbic acid in the blood of hibernating squirrels and observe whether that induces any brain damage.

They hope that such experiments will ultimately lead to a way to curtail the brain cell death that follows a stroke's initial wave of destruction. "Some of the damage [from a stroke] is caused by the lack of oxygen and glucose during the reduced blood flow. Other damage is caused once the blood flow starts again," notes Drew.

This resumption of blood flow, known as reperfusion, apparently exerts fatal stress on some cells as they try to recover from the stroke. To combat this reperfusion-induced damage, physicians might infuse ascorbic acid or another antioxidant into a patient's blood, suggests Rice.

Seeking further clues to how squirrels withstand torpor and their periodic emergence from it, Drew also plans to examine another curiosity concerning hibernation: Reports dating back to the 1950s have noted that the blood of hibernating animals clots slowly.

Drew has confirmed those observations with her arctic ground squirrels. "Their blood didn't clot in 24 hours," she says.

She is now trying to identify the anticlotting factor, or missing clotting factor, that would explain this unusual phenomenon.

The investigators conducting this hibernation research can offer no guarantees that their results will one day help stroke patients, yet they note that the frustrating history of stroke treatment research argues that no potential line of inquiry should be ignored.

"My main interest is clinical. I want to see something end up in an IV bottle and dripping into patients. That's the ultimate goal," says Frerichs. □

Biology

Biological clocks fly into view

Clocks are ubiquitous in today's society. In addition to their traditional homes on mantles, nightstands, and wrists, timepieces now appear on VCRs, computer screens, TVs, microwave ovens, and much more. Nature's fondness for clocks may be even more impressive, however. Scientists report in the Nov. 28 SCIENCE that the fruit fly harbors biological clocks in its head, thorax, abdomen, leg bristles, wings, antennae, and proboscis. "We were really shocked there were these light-resettable clocks throughout the animal," says Steve A. Kay of the Scripps Research Institute in La Jolla, Calif.

Biological clocks are the internal timekeepers that generate circadian rhythms, the day-long cycles of activities. One clock has been well documented in the brains of many animals. Its rhythm governs several easily observable behaviors such as sleeping, and it has been considered the body's master clock. Yet investigators have found that other tissues seem to maintain their own biological clocks. For example, Jadwiga M. Giebultowicz of Oregon State University in Corvallis and her colleagues have found biological clocks in the testes of moths and in the fruit fly's Malpighian tubules, excretory organs similar to kidneys.

By linking the activity of *per*, a gene that acts as a gear in the fly's biological clock, to genes encoding fluorescent markers, Kay and his colleagues obtained striking visual evidence of daily cyclical activity in almost every fruit fly tissue studied. They could reset each clock by exposing isolated tissues to a new light-dark cycle. Rather than being controlled by the clock in the fly's brain, the other clocks may be governed independently by the day-night cycle, suggests Kay. "The master clock for the fruit fly is sunlight," he proposes.

The new work is "mind-boggling. It suggests that there are circadian oscillators all over," says Michael Menaker of the University of Virginia in Charlottesville, who discovered that the mammalian retina has its own biological clock (SN: 4/20/96, p. 245). Unfortunately, adds Menaker, scientists still have few clues to why most of these clocks exist and whether they indeed act independently or form a hierarchy.

Kay and his colleagues now plan to repeat their fruit fly work in mammals by creating transgenic mice in which clock genes are linked to genes encoding fluorescent markers. They will also explore how the individual fly clocks perceive light. "It looks like the circadian photoreceptor is not going to be a known visual pigment," says Kay. —*J.T.*

Resolving the magnetoreception puzzle

Birds and bees do it. So do turtles and fish. All these creatures seem to discern Earth's magnetic field and use that information to chart migratory paths or perform other navigational tasks. Yet the sensory apparatus that makes such feats possible has remained a mystery.

Some investigators have proposed that iron-rich particles called magnetite, which have been found in the brains of several animals, make possible the detection of weak magnetic fields. A few years ago, a research group led by Michael M. Walker of the University of Auckland in New Zealand reported that rainbow trout have particles that appear to be magnetite and a nerve that responds to shifting magnetic fields (SN: 7/8/95, p. 31). In the Nov. 27 NATURE, the same group now describes behavioral experiments demonstrating that the fish can indeed perceive magnetic fields. Moreover, sensory cells near the magnetitelike particles connect to the nerve that fires when the trout is exposed to a magnetic field.

"This is the first real, concrete evidence that magnetite might actually be the basis of a sensory response," says Charles Rafferty, a magnetoreception investigator at the Electric Power Research Institute in Palo Alto, Calif. —*J.T.*