Inflamed Debate Over Neurotoxin

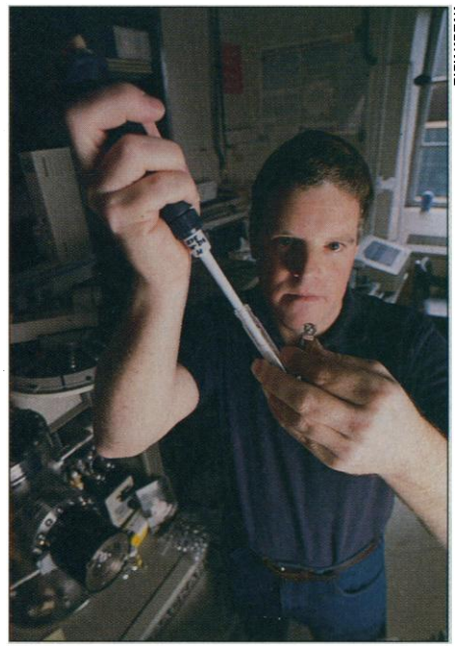
High levels of quinolinic acid have been found in the cerebrospinal fluids of patients suffering from a range of inflammatory brain diseases. Is the chemical cause or effect?

In 1983 Melvin Heyes, then a young postdoc working in Stephen Garnett's laboratory at McMaster University, set out in his girlfriend's car on a 1500-mile trip around Ontario, collecting urine from patients with Huntington's disease. His goal: to test a theory proposed by Robert Schwarcz, a neuroscientist at the University of Maryland Medical School, and neuropathologist William Whetsell, then at the University of Tennessee, that the brain lesions characteristic of Huntington's disease are caused by excess production of a potent neurotoxin called quinolinic acid. But when Heyes returned to his lab with 40 gallons of urine from patients and controls, he was disappointed: Quinolinic acid levels among the Huntington's patients were actually lower than normal.

Most young researchers would have moved quickly on to another topic, but Heyes is nothing if not persistent. He went on to develop a sensitive assay that could measure levels of quinolinic acid directly in cerebrospinal fluid and brain tissue, and he began looking for other neurological disorders in which the neurotoxin—which is present in the normal brain at very low concentrations—might play a role. He has come up with some intriguing findings. Over the past 5 years, Heyes, who is now at the National Institute of Mental Health (NIMH), and collaborators from more than 25 institutions in the United States and Canada have found excess levels of the neurotoxin in the cerebrospinal fluid of patients and laboratory monkeys suffering from a broad range of inflammatory brain diseases. These include HIV-infected patients, Lyme disease patients, and victims of head trauma, stroke, autoimmune diseases, and sepsis. What's more, the excess quinolinic acid levels seem to be linked to difficulty in thinking. Heyes' conclusion: Quinolinic acid may be causing neurological problems by killing or overstimulating NMDA receptors—a known target of quinolinic acid—in the brain. And he believes that the excess quinolinic acid production he and his co-workers have seen is linked to the inflammation process.

If it's correct, Heyes' hypothesis could have important medical implications. If quinolinic acid interferes with cognitive processes, it may eventually be possible to use drugs that block quinolinic acid production to prevent, or even reverse, mental degeneration in patients with inflammatory brain disease. Heyes' conclusions are controversial, however. Many researchers have suggested that quinolinic acid might be a benign marker of disease rather than the cause of neurological impairment. Alternatively, quinolinic acid might be merely one dim star in a constellation of causes, rather than the major culprit. And some neuroscientists such as Dana Giulian at Baylor College of Medicine argue that more research is needed to pin down the source of the quinolinic acid Heyes is detecting.

But Heyes is winning converts following the publication in the October issue of *Brain* of a paper laying out the evidence he and 24



RICK KOZAK

Sensitive assayist. Melvin Heyes.

collaborators have amassed against the neurotoxin. Neuropharmacologist J. David Leander, a research adviser at Lilly Research Laboratories, was among the skeptics. But after reading the *Brain* paper, he told *Science*, "overall, I think it's an amazing scientific puzzle that's being put together by Dr. Heyes and his co-investigators. I have seen this progress from a tentative hypothesis to what I would consider more of an opus." Adds neurologist Joseph B. Martin of the University of California, San Francisco (UCSF), "The extraordinarily high levels of the substance that occur" are very convincing.

It was Schwarcz, says Heyes, who first "opened up the field of quinolinic acid toxicity." As a postdoctoral researcher in Joseph Coyle's laboratory at Johns Hopkins University in the 1970s, Schwarcz was working on animal models for Huntington's disease. He reasoned that whatever causes Huntington's probably exists in small quantities in the normal brain and is produced in excess during illness, and he began to focus on quinolinic acid after reading a paper describing the ability of the neurotoxin to excite nerve cells in the rat cortex. Schwarcz tried injecting the compound into the striatum of rats, and he seemed to have hit pay dirt: The same nerve cells that die in Huntington's patients succumbed, and the animals developed behavioral abnormalities—twitches and slowness of movement—analogous to those seen in Huntington's patients.

Some of the excitement dimmed, however, when Heyes' sensitive assay confirmed the negative results he obtained from urine samples. Other investigators have also failed to find excess quinolinic acid in Huntington's patients. Schwarcz argues, however, that this "does not necessarily invalidate the quinolinic acid hypothesis for Huntington's because it's possible that metabolically compromised neurons are vulnerable to normal concentrations of quinolinic acid." He and other researchers are continuing to investigate the neurotoxin's role in neurodegenerative diseases.

Heyes cast a wider net. His work on quinolinic acid in inflammatory brain disorders began to attract attention early last year when Heyes and Bruce Brew, then of Memorial Sloan Kettering Cancer Center, published a paper in *Annals of Neurology* reporting on a study of more than 100 HIV-infected patients. The researchers found that the concentration of quinolinic acid in the patients' cerebrospinal fluids ranged from 10 to 1000 times normal, and that the degree of dementia observed in these patients correlated with the quinolinic acid levels. Since then, Heyes and his collaborators have found elevated quinolinic acid concentrations in every inflammatory brain disease they have studied, regardless of whether the cause was bacterial, viral, parasitic, fungal, autoimmune, or traumatic.

These results, which were detailed in the *Brain* article, suggest to Heyes that some factor in the inflammation process is stimulating quinolinic acid production in the brain. He has some clues to what that factor may be. In the 1 May issue of *Biochemical Journal* Heyes and Kuniaki Saito, a visiting fellow at NIMH, reported that cultured macrophages, which

Quinolinic Acid's Modus Operandi

Several observations have suggested how excess levels of quinolinic acid might damage neurons. So far, however, these results are limited to in vitro studies, says Madeleine Price, professor of neurobiology and psychiatry at Washington University School of Medicine. The neurotoxin, which is known to bind to NMDA receptors, may overstimulate the neurons, says Price. This would open ion channels, flooding the neurons with sodium and water while disabling the ion pumps that maintain equilibrium. Alternatively, a delayed toxicity may result when quinolinic acid depolarizes the NMDA receptors, and calcium flows into the neurons. This activates proteases, which may somehow cause the damage. Melvin Heyes, a neuroscientist at the National Institute of Mental Health who developed a sensitive assay for quinolinic acid, believes that the neurotoxin may also interfere with cognitive processes by jamming the receptors without killing the neurons.

As for where the excess quinolinic acid comes from, Heyes argues that it is one end product of a six-step metabolic pathway that begins with the amino acid tryptophan. Normally the pathway is all but inactive, but Heyes suggests that gamma interferon, produced when the immune system is activated, turns on the enzyme indolamine 2,3-dioxygenase, which catalyzes the first step in the pathway.

Although Heyes' theory has yet to be proven, he argues that it would explain why, when HIV-infected patients take the drug AZT, quinolinic acid levels drop, and cognitive processes improve. AZT, Heyes points out, inhibits viral reproduction, and the drop in quinolinic acid may be a result of reduced immunological response.

–D.H.

invade the brain during inflammatory brain diseases, can produce more than enough quinolinic acid in vitro to account for levels of the compound that his assay has found in patients' cerebrospinal fluid. And in a paper published in the September *Journal of Neuroimmunology*, Heyes and Brew, now at the University of New South Wales in Sydney, report that the rise in quinolinic acid seems to be proportional to activation of the immune system. They argue that gamma interferon, which is released during immune stimulation, plays a key role in the metabolic pathway that converts the amino acid tryptophan to quinolinic acid (see box).

Schwarcz offers an alternative explanation. He has shown that astrocytes are the only source of quinolinic acid in normal brains. And since these cells play an important role in inflammatory processes as a target and source of cytokines, he argues that they may be a source of the quinolinic acid Heyes and his collaborators have detected. And Giulian, whose own research has failed to show quinolinic acid production by macrophages in culture, suggests that the neurotoxin may be produced in the liver and cross the blood-brain barrier.

Some researchers are also quick to point out that Heyes has not yet proven that quinolinic acid is the major cause of the neurological problems associated with inflammatory brain disease. Giulian argues, for example, that no excess quinolinic acid is found in some diseases, such as Alzheimer's, that involve chronic inflammation. Others, such as neurologist Richard Price of the University of Minnesota, who contributed the samples of cerebrospinal fluid from AIDS patients while at Memorial Sloan Kettering Cancer Center that Heyes assayed in his HIV study, suggest that other neurotoxins may be just as significant. Quinolinic acid, he says, is "probably not the only substance involved, and indeed, it remains to be seen whether it is the most important one." And Heyes collaborator John Halperin, chairman of the department of neurology at North Shore University Hospital in Manhasset, says that although a slight mental slowing down among mildly afflicted Lyme patients "correlates crudely" with a mild elevation of quinolinic acid, other compounds as yet unidentified could be involved in pathologies of severely afflicted patients.

Heyes responds that "they are absolutely right. The quinolinic acid finding has never implied that there are no other factors. But the burden of proof is on identifying those compounds, not on me to refute their hypothetical existence."

Although the hypothesis still remains to be proven, some researchers are intrigued by the potential for developing new therapies to control, treat, or reverse neurological impairments. NMDA receptor blocking compounds, which prevent the binding of quinolinic acid, have been developed, notes UCSF's Martin, and a few are already being tested by pharmaceutical companies. "The possibility of blocking this pathway with pharmaceutical agents is real and should be put to experimental tests," he says.

–David Holzman

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MATHEMATICS

If You Can't See It, Don't Believe It...

Looks can be deceptive—especially when what you're trying to picture exists not in the familiar world of planes or three-dimensional space but in the abstract realm of higher dimensions. There, where looks are limited to what the mind's eye can see, mathematicians have tended to set their compass by what they know is true in two- and three-dimensional space. Often enough, those intuitions are valid in higher dimensions as well. But not always.

Trying to generalize from plane and solid geometry to higher-dimensional space can get you into trouble, as Jeff Lagarias and Peter Shor at AT&T Bell Laboratories in Murray Hill, New Jersey, have shown by putting the kibosh on a proposal, known as Keller's conjecture, about the possible ways of filling space with n-dimensional building blocks. Likewise, Jeff Kahn at Rutgers University and Gil Kalai at the Hebrew University in Israel found higher-dimensional counterexamples to a statement known as Borsuk's conjecture, which sought to generalize the obvious fact that if you break a stick in two, both pieces are shorter than the original. Higher-dimensional spaces, it seems, aren't just amplified versions of the geometries we know well; they are strange lands with their own customs.

That bodes ill for other efforts to understand what life is like in higher dimensions by drawing analogies with the lower-dimensional spaces in which we are at home. The results show "we have very little intuition as to what's going on in high dimensions," Shor admits. The implications go beyond abstract mathematics, notes Joel Spencer, a mathematician at the Courant Institute in New York City. Higher-dimensional geometry may sound abstruse, but mathematicians venture into it whenever they wrestle with a problem involving many variables—and such problems abound in science, from modeling economic activity to analyzing DNA sequences. "Each dimension represents a variable," Spencer explains. Thus, for example, a mathematical model of the economy based on 100 variables "lives" in 100-dimensional space. So trying to visualize what goes on in such a space "is hardly just an exercise of the mind," Spencer says.

But it's not all bad news, the researchers say. The counterexamples that disprove the two conjectures are based on potentially useful properties of higher-dimensional space, properties that geometers simply aren't ac-