Elusive Amylin

A perplexing protein may muddle insulin's message

By KATHY A. FACKELMANN

Biochemist Garth J.S. Cooper believes a protein called amylin underlies Type II diabetes. If he's right, drugs that block amylin might someday offer a new treatment for millions of people with the disease. If he's wrong, his theory will at least have sparked some illuminating investigations into this enigmatic protein, whose very existence remained hidden until just a few years ago.

Cooper readily admits he has a special interest in amylin, scientifically and financially. In 1987, while at the University of Oxford in England, he led the research team that discovered the protein in glue-like deposits typically found in the pancreas of Type II diabetics and identified its chemical structure.

Scientists had known of these deposits for years, but assumed they contained solidified insulin – the normally soluble hormone produced by the pancreas that directs cells to sop up glucose, a form of sugar, from the bloodstream. In Type II diabetes, that sugar uptake falters, leaving abnormally high levels of glucose – and sometimes insulin – circulating in the bloodstream.

But Cooper and his colleagues found no insulin in the deposits. Instead, they extracted an unfamiliar protein made up of 37 amino acids. The researchers, who announced their discovery in the December 1987 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (Vol.84, No.23), named the protein "amylin." (The team inadvertently chose an existing name – albeit rarely used – for a type of starch.) They and other researchers commonly term the solid protein deposits found in the diabetic pancreas "amyloid."

In 1989, Cooper left the academic halls of Oxford and helped found Amylin Corp., a pharmaceutical firm in San Diego, to explore the possibility that amylin may represent the missing link in the genesis of Type II diabetes. In last February's issue of DIABETES, Cooper and colleagues reported a rat study showing that amylin, when injected into the bloodstream at high concentrations, suppresses glucose uptake and eventually produces a syndrome resembling human Type II diabetes.

Cooper stops short of calling amylin *the* cause of Type II diabetes, noting that many other factors – such as diet and genetics – may contribute to the disease. But he contends that an excess of amylin circulating in the bloodstream can lead to insulin resistance, in which cells respond only sluggishly to insulin's instructions (SN: 9/16/89, p.184). Most Type II diabetics have insulin resistance, as do some apparently healthy people at risk of developing Type II diabetes. Cooper and others suspect that insulin resistance may precede the full-fledged disease, which typically strikes after age 40.

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Many scientists initially dismissed Cooper's assertion that amylin causes cells to resist insulin's directives, arguing that animal studies involving unnaturally high concentrations of the protein do not establish such a connection. Recently, however, other researchers have uncovered evidence that appears to bolster the controversial claim. Several reports presented in Atlanta last June, at the annual scientific sessions of the American Diabetes Association (ADA), support a possible link between insulin resistance and amylin.

While scientific opinion remains guarded, those findings have prompted many to reconsider amylin's role in Type II diabetes.

"It would be premature to say that amylin plays a role in the cause of Type II diabetes," says endocrinologist Edward S. Horton of the University of Vermont in Burlington. Nonetheless, Horton calls the theory that amylin produces insulin resistance "an interesting hypothesis."

The stakes surrounding that hypothesis are enormous. If excessive amylin does block insulin's action, drug designers might one day create a compound that blocks amylin, thereby allowing cells to respond normally to insulin. Theoretically, such a drug could prevent Type II diabetes, which today afflicts 11 million people in the United States alone. The disease sometimes leads to limb amputation, blindness or stroke.

Moreover, if insulin resistance can lead to Type II diabetes, an amylin-blocking agent might stave off the full-fledged disease. At present, there is no drug treatment for insulin resistance itself.

The potentially huge market for a new drug to treat Type II diabetics and seemingly healthy people with insulin resistance – coupled with the drama of discovering a new protein that may function as a hormone – has set academic and pharmaceutical laboratories abuzz.

"Amylin has been given a magic-bullet role here in the last three years," says Gerold M. Grodsky of the University of California, San Francisco. "Everybody's

gotten very excited about finding out more about it."

New hints that amylin may play a part in sugar metabolism emerged from a study of healthy rats reported at the ADA meeting by Joanne Chou at Peninsula Laboratories, Inc., in Belmont, Calif. She and her coworkers withheld food from five rats for 40 hours, and from six other rats for 20 hours. A third group of nine rats received unlimited access to rat chow for 24 hours. Afterward, Chou says, the fed rats showed higher amylin levels (averaging 9.8 picograms per milliliter of blood plasma) than either group of fasting rats (averaging 6.8 and 7.5 pg/ml respectively).

Chou says the findings suggest that the same pancreatic "beta" cells that secrete insulin also produce amylin in response to the sugar released after eating a meal. Since beta cells help the body utilize sugar, her data are compatible with the idea that amylin, like insulin, somehow influences sugar metabolism.

"After 50 years of knowing that insulin comes from the beta cell, to discover a new molecule being made and secreted by that same cell: That's a mind-blower," says Daniel Porte Jr. of the University of Washington in Seattle. Porte and his colleagues have published similar evidence indicating that beta cells secrete amylin.

Such findings fit with speculations that amylin normally helps fine-tune blood sugar levels by counteracting insulin's action at the cellular level. Insulin tells cells to load up on sugar circulating in the bloodstream; once those cells have stored enough sugar, amylin may serve to shut off insulin's action, Cooper suggests. In healthy people, the beta cells' tight regulation of insulin and amylin might prevent blood sugar from soaring too high or dipping too low, whereas some beta-cell abnormality could lead to an amylin excess, he theorizes. This excess, in turn, might reduce insulin's effectiveness and lead to higher-than-average blood sugar levels — and, for some people, Type II diabetes, Cooper says.

A team at the Sandoz Research Institute in East Hanover, N.J., has come up with its own data linking amylin to diabetes. Douglas A. Young and his colleagues studied 12 healthy rats while the animals received counterbalancing infusions of insulin and glucose to keep their blood sugar levels "clamped" at a baseline of 90 milligrams per deciliter. For an hour, half the rats also received infusions of amylin, and the remaining rats (serving as controls) received saline infusions.

Rats on amylin showed an 85 percent drop in the amount of glucose needed to maintain the baseline blood sugar levels, Young reported at the ADA meeting. This suggests that an excess of amylin indeed blocks insulin's message to cells and produces insulin resistance, he says.

In a separate experiment, the same team gave amylin injections to 12 healthy rats and noted that their blood glucose rose by 45 percent. "I can infuse amylin and make those animals diabetic," Young asserts.

While such research generates excitement, there's a snag to it: To elicit the insulin-blocking effect, Young, Cooper and many other investigators have had to use "industrial" concentrations of amylin — about 1,000 times higher than the tiny amounts of the protein normally circulating in rat or human blood.

"We're really not sure whether or not the anti-insulin effects of amylin really occur at the physiological concentrations circulating in blood," Horton says.

An *in vitro* study described at the ADA meeting, however, offers the first data suggesting that insulin-blocking effects can result from biologically realistic blood levels of amylin.

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David Kreutter and his colleagues at Pfizer Central Research in Groton, Conn., isolated rat muscle cells, placed the cells in a laboratory dish and bathed them in a glucose solution. Then they added insulin and amylin in the scant, "picomolar" concentrations estimated to occur in human and rat blood. The researchers found that the amylin reduced the cells' ability to take up sugar in a petri dish.

"We were able to induce a state of insulin resistance," Kreutter says. "You can induce these effects at physiologically relevant concentrations of amylin."

Kreutter's report does add weight to the amylin argument. But many scientists say they await confirmation of his findings by others. Moreover, they point out that *in vitro* studies using isolated rat cells cannot demonstrate an *in vivo* connection between amylin and diabetes. So far, attempts to establish that link in humans have produced disappointing results.

To show that amylin can cause insulin resistance and high blood sugar in humans, investigators must first demonstrate that Type II diabetics have higher blood levels of amylin than do nondiabetics. Cooper says he has preliminary data showing just that, although he declines to discuss the details. Other researchers seeking naturally elevated amylin concentrations in diabetics have come up empty-handed.

In one such study, researchers led by Peter C. Butler, then at the Mayo Medical School in Rochester, Minn., recruited 10 Type II diabetics and five healthy nondiabetics. The two groups shared similar blood amylin levels after an overnight fast. But when all the volunteers ate an identical meal, the researchers expected the diabetics to show a rise in amylin. Yet once again, they found no difference between the two groups. The Mayo team describes its results in the June DIABETES.

Butler, now at East Carolina University in Greenville, N.C., says the study prompted him to discount amylin's role in insulin resistance, at least for people with well-established Type II diabetes. He admits his group's findings don't rule out the possibility that people with very early diabetes secrete large amounts of amylin, which might cause insulin resistance.

Cooper cites that possibility as one of two theoretical mechanisms for the development of insulin resistance. Early in Type II diabetes — perhaps even before symptoms appear — the pancreatic beta cells secrete excess amylin, causing muscle cells to resist insulin's message, he suggests. And later, as the overworked beta cells become exhausted, both insulin and amylin production may drop off. By that stage, Cooper says, years of amylin surplus have left cell-damaging amyloid in the pancreas.

Most researchers concur that amyloid deposits can wreak havoc in late-stage Type II diabetes. Porte says the gummy deposits almost certainly destroy or damage beta cells — a process that may contribute to insulin resistance in people with Type II diabetes.

Many questions remain, but the feverish pace of amylin research suggests that laboratories may soon have some answers. And whether or not the protein holds the key to understanding and treating Type II diabetes, amylin's well-kept secrets pose a tantalizing challenge. As Young puts it, "I've got a very interesting protein here, and I am trying to find out how it works." □