A Diabetes Potpourri, Part 2

ZACHARY T. BLOOMGARDEN, MD

As promised in last month's column, we will look this month at several more topics addressed in recent lectures, including the relationship between glycoproteins and diabetes complications, the counterregulation of hypoglycemia in diabetes, and some of the factors involved in hypoglycemia unawareness.

Advanced glycation end products and diabetic complications

One of the dilemmas in understanding the biochemical basis of the complications of diabetes is in developing a theoretical framework to explain why such different tissues are all affected. It is clear that the pathophysiology must be based on the elevation in glucose levels, and in reviewing the pathology of various tissues affected by diabetes it is apparent that glycoproteins accumulate in the tissues. Anthony Cerami, Picoweer Institute for Medical Research, gave the Mosenthal lecture on this topic at the October meeting of the ADA New York Downstate Affiliate. He began his studies with the simplest glycoprotein, HbA_{1c}. Early investigations measuring the amount of HbA_{1c} related to total HbA levels over time showed that diabetic mice have a threefold increased rate of this nonenzymatic process. Cerami and his coworkers realized that HbA_{1c} levels could be used as a marker of glycemic control, "an integral of the average of the blood sugar for about a 30-day period." In studying HbA_{1c}, Cerami stated, "we rediscovered a chemistry that was well known to food chemists since the turn of the century. The accumulation of yellow-brown pigment occurs in much the same chemical fashion in tissues as in foods. We're all cooking at 37 degrees. It is an inevitable process, a function of age." He further commented on the complex chemistry of the process in foods, stating, "The color of coffee, the taste, the aroma, is related to glycation to form 700 compounds. . . glucose or any reducing sugar reacts to form a Schiff base with protein, then Amidori products, then complex further reactions leading to production of advanced glycation endproducts (AGEs). We were very concerned with cross-linking because we think that's where the problem lies in diabetes."

In addition to proteins, the other important biological molecule that reacts with glucose is DNA, perhaps explaining the malformation incidence of 6–10% among children of diabetic mothers, three- to fourfold greater than that in the general population and accounting for 40% of perinatal loss. The molecular basis of diabetic teratogenesis has been studied in animal models using embryo transfer into diabetic and nondiabetic mothers to eliminate effect of drugs used to cause diabetes. With average blood glucose levels twice normal, Cerami described more than a doubling of the mutation rate, with increased frequencies of base substitutions, insertions, and deletions.

End-stage renal disease (ESRD) is associated with a 50% 3-year mortality among individuals with diabetes, with increased levels of peripheral vascular disease, coronary disease, amputations, cerebrovascular disease, blindness, bone disease, and neuropathy. Because of the high mortality, this is an important population to be assessed for abnormalities in AGE production. Individuals with diabetes have arterial wall AGE levels twice those in nondiabetics, and those with ESRD have levels twice again as great, primarily because these substances cannot be effectively dialyzed and their levels can only be corrected with renal transplantation. AGE proteins are usually degraded in macrophages. Normally the products of this are cleared in kidneys. Aminoguanidine is a compound that was found almost a decade ago to prevent advanced glycosylation by reacting with the sugar residues attached to proteins to prevent further modifications. In individuals with diabetes treated with this compound there is a fourfold decrease in AGE levels. "AGE really is a very large class of molecules, most of which are unknown. The AGE peptide is very reactive, and will go up to other peptides and cross-link, a process which aminoguanidine prevents."

To confirm the importance of AGE peptides in the complications of diabetes, Cerami and coworkers studied in vitro incubation of glucose with albumin to form AGE peptides, which were then administered experimentally to nondiabetic animals with and without aminoguanidine. Glomerular sclerosis with proteinuria and renal impairment was found with AGE peptide administration, and this was inhibited with aminoguanidine. Cerami also described a study in rabbits involving administration of AGE for 1 month, then a high cholesterol diet for 1 week. The arterial wall in the AGE-pretreated animals showed increased levels of atherosclerosis. Cerami noted that stroke is more common and severe in individuals with diabetes, and presented data from an experimental model where rats were administered AGE peptides and underwent cerebral arterial occlusion.

Zachary Bloomgarden is a practicing endocrinologist in New York City.

Treatment with aminoguanidine decreased the size of the stroke to levels close to those in controls. Cohen et al. (1) have shown that glycated LDL concentrations in diabetic subjects are twice those in people without diabetes and comprise up to 15% of total apolipoprotein B. AGE-modified ApoB-LDL is present in patients with ESRD and shows delayed clearance. In another investigation, treatment with aminoguanidine in this setting lowered LDL by 30%.

Cerami discussed the presence of phospholipid-AGEs in diabetes and in ESRD. These highly reactive compounds can initiate lipid oxidation, contributing to acceleration of atherosclerosis. A number of recent investigations have produced further evidence regarding the importance of AGEs in the atherosclerotic process. Wautier et al. (2) demonstrated that AGEs are present on the surface of erythrocytes and may increase endothelial oxidative stress. Giardino et al. (3) presentedshowed evidence for nonenzymatic glycosylation of intracellular endothelial cell proteins. And Yan et al. (4) suggested that endothelial cell AGE formation can also increase levels of oxidation, potentiating the atherosclerotic process. In concluding his presentation on the subject, Cerami stated, "Not only is glucose a compound that is extremely reactive, but the products can be just as toxic. We should be able to understand better how to interfere with the process and prevent the complications."

Hypoglycemia

Two recent lectures discussed this important topic, with new understandings of its mechanisms and potentially of approaches to its prevention. Harry Shamoon, Albert Einstein College of Medicine, New York, discussed the topic of counterregulation of hypoglycemia in IDDM and NIDDM at the New York Downstate ADA Affiliate meeting. He pointed out that the frequency of hypoglycemia relates directly to the mean blood glucose level. At a mean blood glucose of 90 mg/dl, 10% of blood glucose levels sampled 7 times per day would be predicted to be <54 mg/dl. The average duration of a mild hypoglycemic episode (blood glucose <50 mg/dl) is 2.5–3 h, and approximately 75% of individuals are unaware of any given episode. Data from the Steno Diabetes center suggest that the individual with IDDM will have, on average, 1.8 such episodes per week, or about 3,500 episodes during a lifetime with diabetes. In the individual without diabetes, hypoglycemia causes elevation initially in levels of both epinephrine and glucagon, both of which increase the hepatic glucose output. The delayed recovery from hypoglycemia in IDDM is associated with little increase in hepatic glucose output, as there is almost completely absent glucagon response and diminished epinephrine response. Epinephrine also raises blood glucose levels by decreasing peripheral glucose uptake, both in adipose tissue and in muscle, and it is this mechanism of restoring blood glucose levels that is most important in individuals with IDDM. The mechanism by which insulin treatment decreases both glucagon and epinephrine responses is uncertain. Glucagon levels and responsiveness to hypoglycemia are normal early after development of IDDM, but become impaired after approximately 1 year, although responsiveness to arginine, exercise, and other non-hypoglycemic stimuli remains unchanged. This neither improves nor worsens with intensive insulin treatment. While epinephrine deficiency typically requires many more years to develop, the epinephrine response to exercise remains similarly normal.

John Gerich, Strong Memorial Hospital, Rochester, NY, discussed the topic of hypoglycemia unawareness at a symposium sponsored by Pratt Pharmaceuticals. He pointed out that "the rate limiting factor in controlling diabetes is hypoglycemia and hypoglycemia unawareness." Better control of diabetes "is not merely more and more insulin." In IDDM, of nine recent studies of a total of 3,165 individuals, 363 had severe hypoglycemia at least once annually. Causes include an inappropriate insulin dose, delayed or missed meals, or alcohol or other medications—but these can be identified in only about a third or a half of all cases. Most often, no clear cause of a given episode of hypoglycemia can be determined.

Hypoglycemia unawareness can be defined as the failure of an individual to develop autonomic symptoms of sweating, tremor, hunger, etc., which are normally seen at a blood glucose around 60 mg/dl, before the development of neuroglycopenic symptoms of lethargy and further decrease in cognitive function, usually occurring at glucose levels below 50 mg/dl. Current data shows little relationship between the rate of fall of blood glucose and the symptoms or manifestations of hypoglycemia. This is not a newly described phenomenon. In 1924, Joslin stated, "dangerous hypoglycemia may occur without warning signs," with the first well-documented series of case reports being that of Mattock and Trimble in *JAMA* in 1928. Gerich recalled the personal experiences described by Lawrence in 1941. This renowned British endocrinologist, who himself had diabetes, said, "As years of insulin life go on, I find it the rule that the type of insulin reaction changes." In an early epidemiological survey, Baldonos and Root described 116 patients with severe hypoglycemia, 80% of whom had duration of diabetes over 10 years. Perhaps because of the relatively lesser degree of glycemic control usually achieved at that time, they were not able to show a relationship to average blood glucose, to the dose or type of insulin, or to the presence of complications.

In the early 1960s studies were first performed during which hypoglycemia was produced under controlled circumstances. It is now clear that glucagon release is the primary response, with catecholamine release as a backup mechanism. Around 1975 demonstration of impaired counterregulatory response in IDDM was shown, with documentation of markedly diminished glucagon response in the majority of patients. In the

late 1980s, with the onset of intensive insulin treatment, the increased frequency of severe hypoglycemia garnered more attention, and the phenomenon of altered awareness was recognized as a major problem. Although the question of whether this is more likely to occur with human than with porcine insulin was raised by a number of studies, Shamoon suggested that subsequent investigations suggest that this is not an important factor. (It should be noted that this remains somewhat controversial. Everett and Kerr have recently reviewed the effects of porcine vs. human insulin, and believe that "neither frequency of severe episodes nor mortality from hypoglycemia are increased following a change from animal to human insulin." They do state, however, that "a small number of patients continue to report an alteration in the nature of hypoglycemic warning symptoms following a change in insulin species." For such patients, they do suggest that animal insulin should continue to be made available [5].)

The prevalence of hypoglycemia unawareness is high in IDDM. Of 1,161 patients reviewed in 12 recent studies, 26% showed this abnormality, while in the DCCT analysis the reported prevalence was 51%. Hepburn et al. (6) showed that 18% of those with normal hypoglycemia awareness, but 91% of those with unawareness, had severe reactions annually, although the frequency of milder degrees of hypoglycemia was similar in the two groups. Gerich defined unawareness as being present if symptoms develop at blood glucose more than 2 standard deviations below the level seen in a nondiabetic population. Interestingly, he provided data suggesting that these individuals had lower thresholds of neuroglycopenia, as well as of autonomic symptoms, during a stepped clamp study. Individuals with hypoglycemia unawareness show both longer duration of diabetes and lower glycohemoglobin levels than individuals with normal awareness of hypoglycemia, and these appear to be independent risk factors for the condition.

Several potential mechanisms exist for hypoglycemia unawareness. Target tissue responsiveness to catecholamines could be diminished. This has been shown not to be the case, as the reduced catecholamine response is selective for hypoglycemia, with patients showing exaggerated response to other stimuli, such as exercise. Decreased release of autonomic neurotransmitters could be present as a general phenomenon. Autonomic neuropathy, however, is not necessarily associated with diminished epinephrine response to hypoglycemia. There is extensive data suggesting that CNS recognition of hypoglycemia is diminished with hypoglycemia unawareness. Although altered hypothalamic neuronal glucose recognition tissue could explain this, most research suggests that chronic hypoglycemia induces an initially adaptive increase in blood-brain barrier glucose transport, which paradoxically has the effect of diminishing recognition of hypoglycemia. Conversely, with chronic hyperglycemia there is decreased blood-brain barrier glucose transport, which is perhaps related to the hypoglycemic symptoms experienced by some individuals with poor glycemic control, despite normal or only slightly low glucose levels.

Studies have shown that when hypoglycemia is induced repeatedly, there is a decrease in counterregulatory hormone response and in symptoms to subsequent episodes (7). If hypoglycemia is prevented, this cycle can be broken. This has most dramatically been shown in individuals with insulinoma, who demonstrate decreased release of counterregulatory hormones and decreased symptoms of hypoglycemia. Postoperatively, there is an increase toward normal in counterregulatory hormone release, and recognition of symptoms recovers. Three studies have now been done in IDDM showing that, as Gerich put it, "if you lighten up a little bit, people can improve their awareness." Gerich noted that there may be some forms of hypoglycemia unawareness that cannot be reversed, perhaps representing "central neuropathy."

Shamoon concluded by discussing hypoglycemia in NIDDM, about which there is much less information. Jennings et al. (8) reported that about 20% of individuals with NIDDM had at least one hypoglycemic episode, usually mild, during a 6-month period of observation. Hepburn et al. (9) reported, however, that the frequency of severe hypoglycemia in insulin-treated individuals with NIDDM is similar to that in IDDM. The frequency of hypoglycemia in NIDDM has particularly great importance as we consider the application of intensive insulin regimens to insulin-requiring type II patients. An additional question of consequence would be the potential role of β-blockers, which are relatively contraindicated in individuals with substantial risk of hypoglycemia, but which have been shown to offer potential benefit as cardioprotective agents in hypertensive diabetic patients (10). A subtrial occurring within the UKPDS, the Hypertension in Diabetes Study, compares atenolol with captopril and will give additional information on the benefits and potential adverse effects of β-blockers in insulin-treated patients with diabetes.

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