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Oxidative stress and diabetic neuropathy: a new understanding of an old problem

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Diabetes has reached epidemic proportions in the Western world. In the United States, 17 million individuals have diabetes, greater than 6% of the population (1). The morbidity and mortality of diabetes is due to the development of both macrovascular and microvascular complications (2). Macrovascular complications including myocardial infarction, stroke, and large vessel peripheral vascular disease are 2 to 4 times more prevalent in individuals with diabetes. The underlying common factor in macrovascular complications is the ability of the diabetic condition to accelerate atherogenesis. Atherogenesis is a multifactorial response of vessels to injury; both insulin resistance and elevated lipid levels, common in diabetes, are primary triggers of atherogenic injury (3). The endothelium in diabetic arteries is also more prone to atherogenic injury, likely due to decreased production of endothelial nitric oxide, known to be antiatherogenic, and increased production of plasminogen activator inhibitor-1 (PAI-1) (4). While macrovascular complications are common among diabetics, diabetes-specific microvascular complications will eventually affect nearly all individuals with diabetes. Diabetic retinopathy is the most common cause of adult blindness in the United States. Ninety percent of diabetics present evidence of retinopathy within 15 years of disease onset and approximately 25,000 new cases of diabetes-related blindness are reported per year (5). Diabetes is also the leading cause of renal failure in the United States, accounting for 40% of new cases each year (6). Greater than half of all patients with diabetes develop neuropathy, a progressive deterioration of nerves resulting in peripheral and autonomic nerve dysfunction. As a result, diabetic neuropathy is the most common cause of nontraumatic amputations and autonomic failure (7, 8). In his or her lifetime, a diabetic patient with neuropathy has a 15% chance of undergoing one or more amputations (9).

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Nonstandard abbreviations used: plasminogen activator inhibitor-1 (PAI-1); advanced glycation end-product (AGE); nerve growth factor (NGF); neurotrophin-3 (NT-3).

What are the mechanisms that underlie the development of microvascular complications?

Similar to our understanding of macrovascular complications, it is becoming increasingly clear that microvascular complications share a common pathophysiology. Animal and in vitro experiments over the last 25 years have implicated four major pathways of glucose metabolism in the development of microvascular complications (10). These include: 1) increased polyol pathway activity leading to sorbitol and fructose accumulation, NAD(P)H-redox imbalances, and changes in signal transduction; 2) nonenzymatic glycation of proteins yielding advanced glycation end-products (AGEs); 3) activation of PKC thereby initiating a cascade of stress responses, and 4) increased hexosamine pathway flux (1, 2, 10, 11). While specific inhibitors of each pathway block one or more diabetic microvascular complications, only recently has a link been established that provides a unified mechanism of tissue damage. Each pathway becomes perturbed as a direct or indirect consequence of hyperglycemia-mediated superoxide overproduction by the mitochondrial electron transport chain. Either inhibition of superoxide accumulation or euglycemia restores the metabolic and vascular imbalance and blocks both the initiation and progression of complications (2, 10, 12).

In the diabetic state, unchecked superoxide accumulation and resultant increases in polyol pathway activity, AGE accumulation, PKC activity, and hexosamine flux trigger a feed-forward system of progressive cellular dysfunction (Figure 1). In nerve, this confluence of metabolic and vascular disturbances leads to impaired neural function and loss of neurotrophic support, and long term, can mediate apoptosis of neurons and Schwann cells, the glial cells of the peripheral nervous system (13–15). Decreases in nerve growth factor (NGF), neurotrophin-3 (NT-3), ciliary neurotrophic factor, and IGF-I in nerves from animals with experimental diabetes are well documented and correlate with the presence of neuropathy (16–18).

Hedgehog proteins and diabetic neuropathy

The elegant work of Calcutt and colleagues in this issue of the *JCI* reports a decrease in desert hedgehog expression in nerves from young adult rats with streptozotocin-induced diabetes (19). Hedgehog proteins (sonic, desert, and indian) are essential for normal nervous system development (20). Desert hedgehog is found exclusively in the peripheral nervous system in Schwann cells and is important in peripheral nerve patterning (20). After 10 weeks of experimental diabetes, Calcutt et al. observed a decrease in desert hedgehog gene expression. This decrease correlates with several well established physiological and biochemical markers of experimental

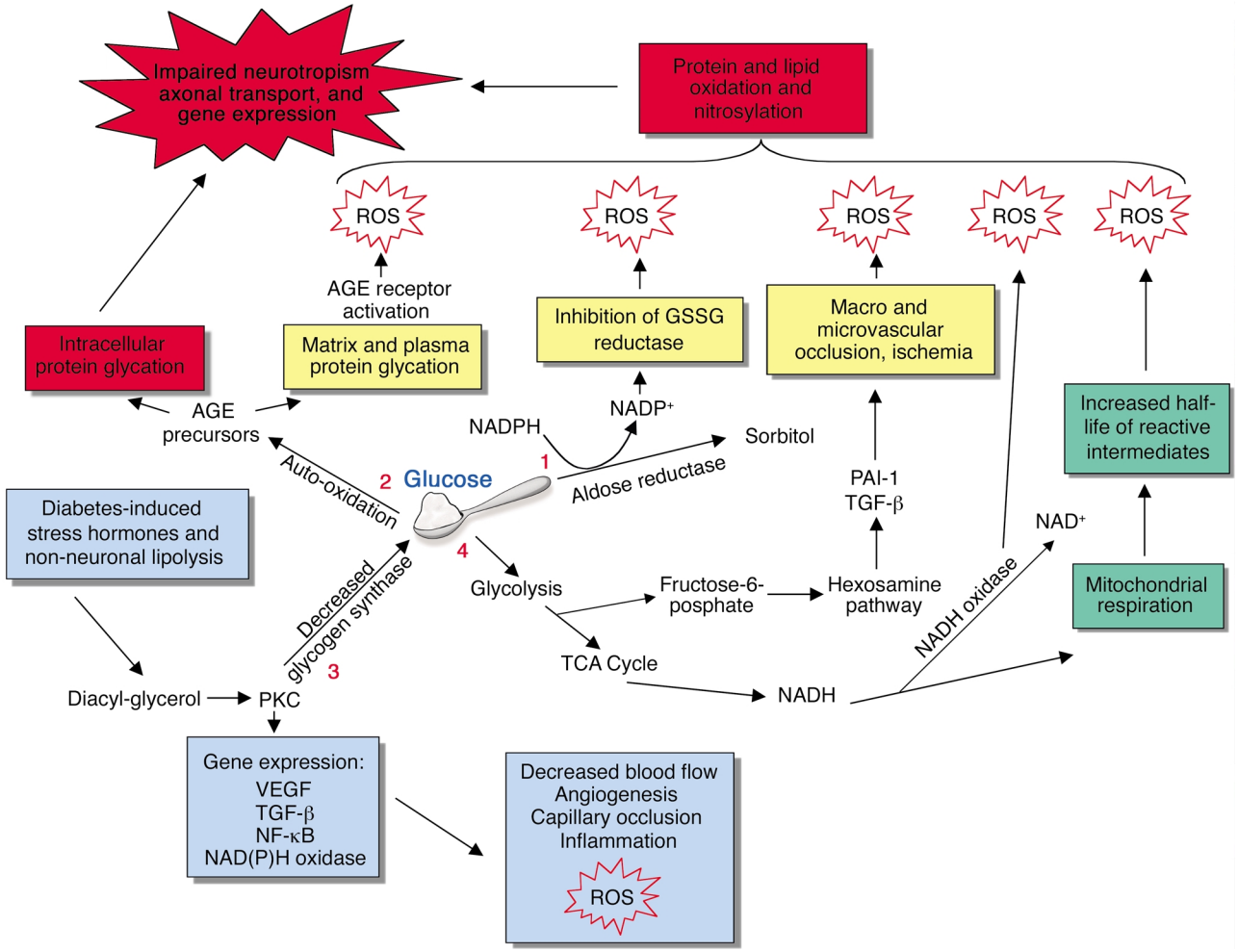

Figure 1
Mechanisms leading to neuronal degeneration in hyperglycemia involve reactive oxygen species (ROS) formation. The diabetic state produces impaired neurotropism, axonal transport and gene expression through at least four major pathways. 1) Excess glucose is diverted away from glycolysis by the polyol pathway that depletes NADPH and cellular antioxidant capacity. 2) Glucose also may become oxidized and form AGEs that alter extracellular matrix, activate receptors that produce ROS intermediates, and alter intracellular protein function. 3) PKC becomes activated either directly by glycolytic intermediates or indirectly as shown as a second messenger for stress hormones, leading to increased vascular disease, inflammation, and oxidative stress. 4) Partial glycolysis causes accumulation of glycolytic intermediates and leads to escape of fructose-6-phosphate along the hexosamine pathway that increases vascular disease and further ROS generation. These mechanisms are ultimately linked to superoxide production through increased glucose respiration that produces superoxide in the mitochondria and also activates the superoxide-producing NADH oxidase. GSSG, glutathione disulfide; TCA, tricarboxylic acid cycle.

diabetes, including slowed motor and sensory nerve conduction velocities, decreased nerve blood flow, decreased pain threshold in response to heat and/or formalin, and decreased NGF and neuropeptide levels. Thrice weekly injections of sonic hedgehog linked to an IgG fusion protein, beginning after 5 weeks of experimental diabetes and continuing for an additional 5 weeks, restored motor and sensory nerve conduction velocities and both NGF and neuropeptide levels. There was no therapeutic effect on nerve blood flow or pain threshold levels. Morphometric analyses of sciatic nerves revealed that diabetic animals had a decrease in medium sized myelinated fibers, which was restored by sonic hedgehog treatment.

Combination therapy for the treatment of diabetic neuropathy

While purely speculative, it is likely that restoration of hedgehog activity provided much needed neurotrophic support both directly, by activating hedgehog downstream pathways and indirectly, by restoring NGF levels. As discussed above, hyperglycemia-induced decreases in neurotrophic factors are well documented, with neurotrophic replacement frequently restoring one or more impaired nerve parameters to normal. Administration of NGF restores neuropeptide levels and sensory amplitudes in experimental diabetes (17, 21); in parallel, NT-3 normalizes nerve conduction slowing (22, 23) and IGF-I administration blocks the development of neuropathy and reverses impaired nerve regeneration (24, 25). When oxidative stress is induced in nerves of nondiabetic animals by administering pro-oxidants, decreases in NGF and NT-3 are observed similar to those reported in animals with experimental diabetes (26). Antioxidant therapy in experimental diabetic neuropathy blocks the observed decreases in nerve NGF and

restores nerve function (27). Antioxidant therapy also restores normal blood flow and nerve conduction velocities in experimental diabetes (11, 12, 28–32). Interestingly, neurotrophic factors may also serve as antioxidants and this function may contribute to their role as possible therapeutic entities in diabetic neuropathy (33–36).

Currently, there are no treatments for neuropathy other than treating the diabetic condition per se. Our improved understanding of the pathogenesis of diabetic neuropathy should assist in the development of new therapies (7, 8, 10). Clearly, therapeutic efficacy in man will be more challenging than in animal models of experimental diabetes. Damage to the nervous system by diabetes in man is more chronic, extensive, and severe (7, 8, 10). Several clinical trials have already failed to show improvement of diabetic neuropathy in patients with type 1 and 2 diabetes (reviewed in ref. 1). For example, there is no therapeutic benefit of acetyl-carnitine, aldose reductase inhibitors, or NGF in human diabetic neuropathy (1). What is required is combination therapy. Similar to how the oncologist approaches cancer, the endocrinologist and/or neurologist could, in the future, approach diabetic neuropathy. By blocking multiple pathway components (Figure 1), multiple causes of oxidative stress would in turn be blocked, preventing nervous system injury. If coupled with additional antioxidant therapy or neurotrophic support, this type of "complication cocktail" could provide the first effective treatment for diabetic neuropathy.

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