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Contribution of Polyol Pathway to Diabetes-Induced Oxidative Stress

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Abstract. Diabetes causes increased oxidative stress, which is thought to play an important role in the pathogenesis of various diabetic complications. However, the source of the hyperglycemia-induced oxidative stress is not clear. It was found that the polyol pathway is the major contributor to oxidative stress in the lenses and nerves of diabetic mice. The first enzyme in the pathway, aldose reductase (AR), reduces glucose to sorbitol, which is then converted to fructose by sorbitol dehydrogenase (SDH). Transgenic mice that overexpress AR specifically in their lenses showed a significant increase in oxidative stress when they became hyperglycemic, as indicated by a decrease in GSH and an increase in malondialdehyde in their lenses. Introducing an SDH-deficient mutation into these transgenic mice significantly normalized the GSH and malondialdehyde levels. These results indicate that both enzymes of the polyol pathway contributed to hyperglycemia-induced oxidative stress in the lens. In the wild-type mice, diabetes caused a significant decrease in GSH in their sciatic nerves, indicative of oxidative stress. In the AR null mutant mice, diabetes did not lead to any decrease in the nerve GSH level. These results indicate that similar to the situation in the lens, AR is also the major contributor to hyperglycemia-induced oxidative stress in the nerve. Although increased flux of glucose through the polyol pathway leads to diabetic lesions in both the lenses and nerve, the mechanisms may be different. AR-induced osmotic stress seems to be the cause of diabetic cataract, whereas AR-induced oxidative stress is probably the cause of neuronal dysfunction.

Diabetes causes increased oxidative stress in various tissues as evidenced by increased levels of oxidized DNA, proteins, and lipids. Besides damaging the functions of these molecules, oxidative stress also triggers a series of cellular responses, including the activation of protein kinase C (PKC) (1,2), transcription factor NF-κB (3), and JNK stress-associated kinases (4), and so forth. Inappropriate activation of these important regulatory molecules would have deleterious effects on cellular functions, and it is thought to contribute to the pathogenesis of various diabetic complications (5). However, it is not clear how hyperglycemia leads to increased oxidative stress. It is most likely the combined effects of increased levels of reactive oxygen species (ROS) and decreased capacity of the cellular antioxidant defense system. Glucose auto-oxidation (6), nonenzymatic glycation (7), and the interaction between glycated products and their receptors (8), overproduction of ROS by mitochondria (9), and the polyol pathway (10,11) all are potential sources of hyperglycemia-induced oxidative stress. This report focuses on the contribution of the polyol pathway to oxidative stress.

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The polyol pathway consists of two enzymes. The first enzyme, aldose reductase (AR), reduces glucose to sorbitol with the aid of its co-factor NADPH, and the second enzyme, sorbitol dehydrogenase (SDH), with its co-factor NAD^+, converts sorbitol to fructose. In animal models, treatment with AR inhibitors (ARI) was shown to be effective in preventing the development of various diabetic complications, including cataract, neuropathy, and nephropathy (12). It was thought that osmotic stress, from the accumulation of sorbitol, leads to diabetic lesions (13). Although this model may be applicable to the lens, in other tissues, such as sciatic nerve, the level of sorbitol does not correspond to the severity of neural dysfunction (14), suggesting that other mechanisms may be more important in contributing to diabetic lesions. Treatment of diabetic rats with an ARI attenuated the reduction of GSH in their lenses, suggesting that AR activity causes oxidative stress (15). However, the ARI may have free radical scavenging function; therefore, the normalization of GSH may not be due to the inhibition of AR (16). Here, we report the use of a genetic approach to demonstrate that both AR and SDH contribute to diabetes-induced oxidative stress.

Polyol Pathway and Diabetes-Induced Oxidative Stress in the Lens

Mice have low levels of AR in their lenses, and they are resistant to develop diabetic cataract. To determine the role of AR in the pathogenesis of cataract, we developed transgenic mice that overexpress the human AR cDNA specifically in their lenses. Expression of the AR transgene was found only in

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the lens and no other tissues. Under normal rearing condition, no morphologic abnormality was detected in the lenses of the transgenic mice, indicating that overexpression of AR *per se* does not have any deleterious effect on the lens. When induced to become diabetic by streptozotocin injection, the transgenic mice developed cataract at a rate proportional to the level of AR expression in their lenses, indicating that AR is the key enzyme in the pathogenesis of diabetic cataract (17).

These lens-specific AR transgenic mice were used to determine whether the polyol pathway activity contributes to diabetes-induced oxidative stress (18). When the wild-type mice were induced to become diabetic, their lenses showed no sign of experiencing oxidative stress. However, the lenses of diabetic transgenic mice had significant decrease in GSH level and significant increase in the level of malondialdehyde (MDA), indicative of oxidative stress (Figure 1). These results indicate that AR is the major contributor to diabetes-induced oxidative stress in the lens. Introducing a copy of the SDH-deficient mutation into the AR transgenic mice partially normalized the GSH and MDA levels in the diabetic transgenic mice, indicating that SDH also contributes to oxidative stress (Figure 1).

Polyol Pathway and Diabetes-Induced Oxidative Stress in the Nerve

Wild-type mice are susceptible to develop diabetic neuropathy as indicated by reduced nerve conduction velocity (NCV) and signs of structural abnormality of the nervous tissues (19). To determine the role of polyol pathway in the pathogenesis of this disease, we developed AR gene knockout mice (20). The growth rate and reproductive capacity of these mice were similar to that of the wild-type mice. The only observable abnormality in the AR-deficient mice is that they drink and urinate more than the wild-type mice, indicating a mild impairment in their urine concentrating ability. However, this does not affect the levels of various electrolytes in their serum.

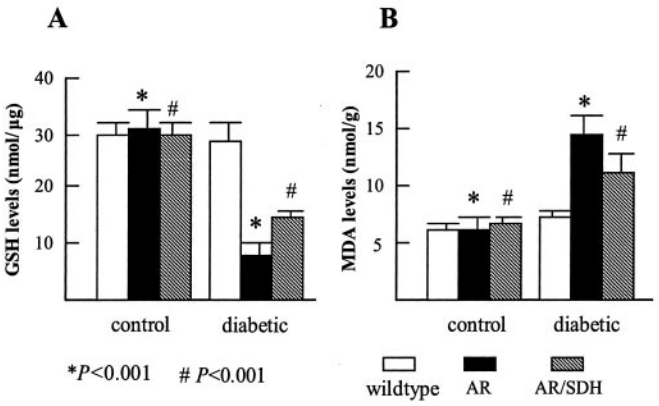

Figure 1. Polyol pathway–induced oxidative stress in diabetic lens. GSH (A) and MDA (B) of wild-type, AR (heterozygous CAR648 AR transgenic), and AR/SDH (heterozygous CAR648 AR transgenic and heterozygous SDH-deficient double mutant) mice under normal and diabetic conditions. The bars indicate mean ± SD. The *P* values were calculated by *t* test.

When these mice were induced to become diabetic, they showed no reduction in their NCV, indicating that AR deficiency confers to these mice resistance to develop diabetic neuropathy (Figure 2). Whereas the diabetic wild-type mice showed significant reduction in the GSH level in their sciatic nerve, diabetic AR null mice showed no change in the GSH level, indicating that the polyol pathway is the major source of diabetes-induced oxidative stress in this tissue.

Discussion

We have shown that the polyol pathway is the major source of diabetes-induced oxidative stress in lens and the nerve. There are three potential mechanisms for the polyol pathway to contribute to oxidative stress (Figure 3). (*1*) AR activity depletes its co-factor NADPH, which is also required for glutathione reductase to regenerate GSH. Under hyperglycemic condition, as much as 30% of the glucose is channeled into the polyol pathway (10), causing a substantial depletion of NADPH and consequently a significant decrease in the GSH level. Thus, during hyperglycemia, AR activity diminishes the cellular antioxidant capacity. (*2*) Oxidation of sorbitol to fructose by SDH causes oxidative stress because its co-factor NAD^+ is converted to NADH in the process, and NADH is the substrate for NADH oxidase to generate ROS (21). (*3*) The polyol pathway converts glucose to fructose. Because fructose and its metabolites fructose-3-phosphate and 3-deoxyglucosone are more potent nonenzymatic glycation agents than glucose, the flux of glucose through the polyol pathway would increase advance glycation end products (AGE) formation. AGE, as well as binding of AGE to their receptors, are known to cause oxidative stress.

Although the polyol pathway causes oxidative stress in both the lens and the nerve, its role in the development of diabetic lesion in these two tissues seemed to be different. Osmotic stress, from the accumulation of sorbitol, is a more important factor for the development of diabetic cataract. This was demonstrated by the fact that administration of vitamin E and vitamin C, even though significantly normalized GSH and MDA levels in the diabetic lens, could not prevent the development of cataract. It only delayed the onset of cataract for a couple of days (18). Furthermore, blocking the conversion of sorbitol to fructose by SDH mutation, which led to higher level of sorbitol accumulation and reduced oxidative stress, exacerbated cataract development (17). Taken together, these results strongly indicate that osmotic stress is the major contributing factor in diabetic cataract development in this experimental model in which cataract develops in a matter of weeks. This model simulates the acute diabetic cataract in patients with uncontrolled hyperglycemia. In patients with diabetes and moderately well-controlled blood glucose level, cataract may take >10 yr to develop. It is likely that in the slow-developing diabetic cataract, chronic oxidative stress may be a more important factor. In the nerve, although the level of sorbitol is increased during hyperglycemia, it is most likely not the cause of diabetes-induced functional impairment. The sorbitol level in the nerve of nondiabetic SDH-deficient mice is higher than that of diabetic wild-type mice (14), yet the NCV of the

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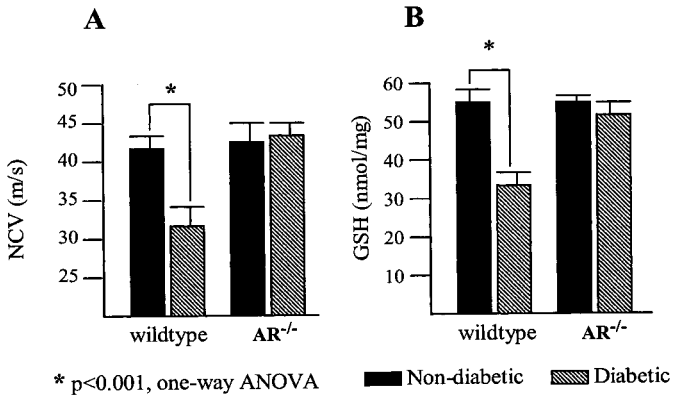

Figure 2. AR in diabetic neuropathy. NCV (A) and GSH (B) levels of wild-type and AR−/− (homozygous AR null mutant) mice under normal and diabetic conditions. The bars indicate mean ± SD. The *P* values were calculated by one-way ANOVA.

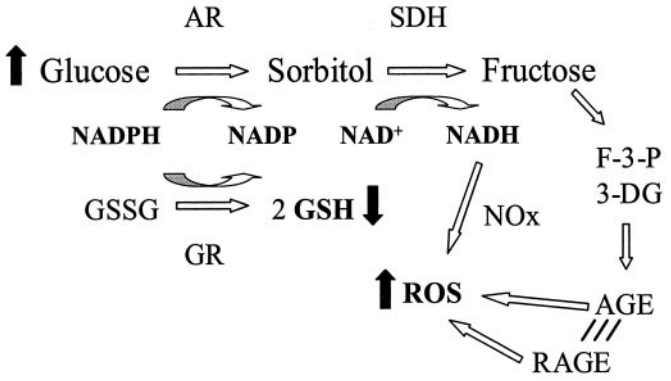

Figure 3. Polyol pathway–induced oxidative stress. AR competes with glutathione reductase (GR) for their co-factor NADPH, leading to a decrease in GSH. Increased NADH causes NADH oxidase (NOx) to produce ROS. Fructose-3-phosphate (F-3-P) and 3-deoxyglucosone (3-DG), metabolites of fructose, increase AGE formation. AGE and binding of AGE to receptor of AGE (RAGE) increase oxidative stress.

nondiabetic SDH-deficient mice is normal, indicating that a higher level of sorbitol alone does not cause any damage to the nerve. Polyol pathway–induced oxidative stress is most likely an important contributing factor to diabetic neuropathy. This is supported by a number a studies that showed that antioxidant treatment significantly attenuated some of the symptoms of this disease (22–24).

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