Are Disturbances of Sorbitol, Phosphoinositide, and Na^+-K^+-ATPase Regulation Involved in Pathogenesis of Diabetic Neuropathy?

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Alterations in *myo*-inositol and phosphoinositide metabolism, induced by hyperglycemia and prevented by aldose reductase inhibitors, are implicated in impaired Na^+-K^+-ATPase regulation in peripheral nerve and other tissues prone to diabetic complications by an increasing range of scientific observations. However, the precise role of these related metabolic derangements in various stages of clinical complications is complex. For instance, it appears that these biochemical defects may play a role not only in the initiation of diabetic neuropathy but also in its later progression. Therefore, full appreciation of the potential pathogenetic role of altered phosphoinositide metabolism in diabetic complications requires detailed studies of both the earliest and the more mature stages of these disease processes. *Diabetes* 37: 688–93, 1988

A unifying metabolic hypothesis completely accounting for the development of one or more of the chronic complications of diabetes on the basis of a single disturbance of glucose metabolism attributable to insulin deficiency and/or hyperglycemia has long been sought by interested clinical and basic scientists. However, neuropathy and other long-term complications of diabetes are generally attributed to the interaction of multiple metabolic, genetic, and environmental factors, although hyperglycemia and/or insulin deficiency appears to exert a permissive influence. A growing body of internally consistent scientific data obtained from cultured cells, incubated tissue preparations, animal models, and clinical studies implicate glucose-induced disturbances of sorbitol, *myo*-inositol, and phosphoinositide metabolism and Na^+-K^+-ATPase regulation in a series of secondary biochemical, functional, and architectural abnormalities in the peripheral nervous system in diabetes. In some cases, these early glucose-induced sorbitol- and *myo*-inositol–related functional and structural changes resemble those characteristic of human diabetic neuropathy.

Chronic hyperglycemia and/or insulin deficiency are implicated in the pathogenesis of diabetic neuropathy by several generally accepted observations. *1*) The most consistently described morphological picture of combined degeneration of small and large myelinated axons and segmental demyelination and remyelination characteristic of chronic diabetes is nonspecific, resembling that of other "metabolic" neuropathies (1). *2*) The prevalence of diabetic neuropathy is similar in insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) diabetes mellitus despite their disparate underlying pathogenesis (1). *3*) The prevalence of diabetic neuropathy (corrected for duration of diabetes) is higher in patients whose chronic diabetes has been more poorly controlled (1). *4*) Objective measures of subclinical neuropathy (slowed nerve conduction or impaired autonomic or sensory function in the absence of clinical signs or symptoms of diabetic neuropathy) in both diabetic patients and animal models parallel the severity and/or duration of hyperglycemia and/or insulin deficiency. For example, it has been known for over 20 years that motor nerve conduction velocity is slightly reduced at diagnosis of IDDM but improves rapidly with, and declines rapidly without, insulin-replacement therapy. The pattern of change suggests an initial direct and reversible metabolic contribution to motor nerve conduction slowing in newly diagnosed diabetes (2,3). This initial electrophysiological response is accompanied by improvement in vibratory perception threshold (4). Nerve conduction velocity slows progressively but modestly with the duration of chronic diabetes (5,6). The preponderance of conduction slowing in clinically established (and probably also chronic subclinical) diabetic neuropathy is accounted for by a loss of large myelinated fibers and therefore is slow to reverse (7). Motor nerve conduction velocity improves

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slightly but proportionately with glycosylated hemoglobin (HbA_{1c}) in response to metabolic therapy in chronic stable NIDDM (8) and IDDM (9–15). Thus, improvement of nerve conduction velocity after acute metabolic correction of chronic diabetes is necessarily confined to the small component of conduction slowing not attributed to nerve fiber loss. However, this portion of nerve conduction slowing in diabetic patients can reverse rapidly with metabolic therapy and therefore probably reflects a direct biochemical or biophysical contribution related to metabolic, not structural, abnormalities in peripheral nerve.

When most researchers attributed the slowing of nerve conduction in diabetes entirely to fiber degeneration or demyelination rather than to a potentially reversible metabolic disorder, some researchers explored the basis for the rapidly reversible component of nerve-conduction slowing in various spontaneously occurring and experimentally induced diabetic animal models to identify putative metabolic mediators of nerve damage in diabetes (16–29) and explored the importance of hyperglycemia in the pathogenesis of the complications of diabetes (30). Altered nerve *myo*-inositol metabolism was first examined as a possible mediator of the effects of hyperglycemia on nerve conduction in experimental diabetes over a decade ago (17), when the important role of *myo*-inositol and its metabolites in intracellular regulation was largely unrecognized. Two weeks of untreated streptozocin-induced diabetes (STZ-D) in the rat decreased both motor nerve conduction velocity and the levels of water-soluble *myo*-inositol in the sciatic nerve without affecting plasma *myo*-inositol concentration. Insulin therapy that restored normal growth and well-being but only partially corrected hyperglycemia failed to improve either sciatic motor nerve conduction velocity or *myo*-inositol levels, whereas intensive insulin therapy that controlled hyperglycemia normalized both conduction and *myo*-inositol (17). Pharmacologic dietary *myo*-inositol supplementation administered to otherwise untreated diabetic and normal rats raised plasma *myo*-inositol levels six- to sevenfold and eliminated the difference in sciatic nerve *myo*-inositol content between normal and diabetic rats; sciatic motor nerve conduction velocity, unaffected in the nondiabetic rats, was normalized in the STZ-D rats despite unabated hyperglycemia and elevated sciatic nerve glucose, sorbitol, and fructose levels (17). This and other confirmatory studies in both experimentally induced (18,31) and spontaneous (25) diabetes in the rat suggested a role for hyperglycemia-induced nerve *myo*-inositol depletion in the slowing of nerve conduction in diabetes.

Independent interest in the possible pathogenetic role of the markedly increased reduction of excess glucose or galactose to its polyol derivative (sorbitol or dulcitol, respectively) by aldose reductase in response to hyperglycemia in tissues such as nerve (where hexose entry is neither rate limiting for metabolism nor primarily modulated by insulin; 32) led to the development of highly specific and potent inhibitors of aldose reductase (33). Such inhibitors not only prevent the accumulation of polyol intermediates in the lens and peripheral nerve of diabetic and galactose-intoxicated rats (33) but also duplicate the effects of 1% dietary *myo*-inositol supplementation on both motor nerve conduction velocity and nerve *myo*-inositol content in the STZ-D and spontaneously diabetic BB rat (25,28,31,34–38). Furthermore, aldose reductase inhibitors prevent *myo*-inositol depletion and/or duplicate effects of *myo*-inositol supplementation in other tissues prone to develop diabetic complications, i.e., the retina and the renal glomerulus in some rodent species (39–43). [Nerve *myo*-inositol is either not (44) or only marginally (34) depleted in the diabetic mouse, which does not accumulate sorbitol in peripheral nerve (34,44), perhaps because of abnormal characteristics or distribution of its polyol-pathway enzymes.] Thus, polyol-pathway activation induced by hyperglycemia appears to be primarily responsible for *myo*-inositol depletion in peripheral nerve and other tissues prone to diabetic complications. Furthermore, the effects of polyol-pathway activation in diabetic nerve on impulse conduction appear to be mediated by the accompanying depletion of tissue *myo*-inositol (45,46). Yet, extensive in vivo and in vitro studies of composite peripheral nerve and transformed cells of neural origin provide only a partial understanding of the effects of glucose, sorbitol, insulin, and experimental diabetes on nerve *myo*-inositol and phosphoinositide metabolism (47–58).

The content of phosphoinositide, much of which is sequestered in relatively metabolically quiescent myelin, is inconsistently altered in experimental diabetes (48–50,59). The activities of both CDPdiacylglycerol–inositol phosphatidyltransferase, the rate-limiting enzyme for phosphatidylinositol synthesis, and phosphatidylinositol-4-phosphate kinase are diminished in experimental diabetes (51,52), yet the pattern of incorporation of radiolabeled *myo*-inositol or orthophosphate into nerve phosphoinositides is not altered in a consistent pattern (50,52,55,60,61). Peripheral nerve expresses a high-affinity sodium-dependent transport system for *myo*-inositol that is inhibited by glucose at concentrations present in the plasma of diabetic patients (47,54) and apparently is not acutely stimulated by physiologic concentrations of insulin (47). Experimental diabetes diminishes nerve *myo*-inositol uptake beyond that which can be accounted for by the persistence of elevated glucose levels (53,54), possibly due to retained tissue sorbitol (54) or impaired ion pumping (53).

Neuroblastoma cells grown in a high level of glucose for 2 wk exhibit decreased *myo*-inositol uptake and *myo*-inositol and phosphatidylinositol content that is prevented by pretreatment with an aldose reductase inhibitor and duplicated by culture with 1 mM sorbitol (56). If confirmed, these studies would suggest that sorbitol interferes with *myo*-inositol uptake and may account for the effects of elevated glucose levels on *myo*-inositol metabolism in this cell type.

A provocative preliminary study by Dunlop et al. (57) of N1E-115 neuroblastoma cells raises the possibility that exposure to high levels of glucose induces insulin modulation of *myo*-inositol transport. Taken together, the studies indicate that composite *myo*-inositol metabolism in peripheral nerve is altered in a complex fashion by diabetes by several potential mechanisms, including direct and indirect effects of glucose or its metabolite sorbitol on *myo*-inositol transport, which are possibly complicated by secondary effects of disturbed ion pumping or insulin action.

The evidence that slowing of nerve conduction in acute experimental diabetes results from reversible disturbances in *myo*-inositol and phosphoinositide metabolism caused by insulin deficiency and hyperglycemia prompted a search for an underlying biophysical and/or metabolic mechanism. The

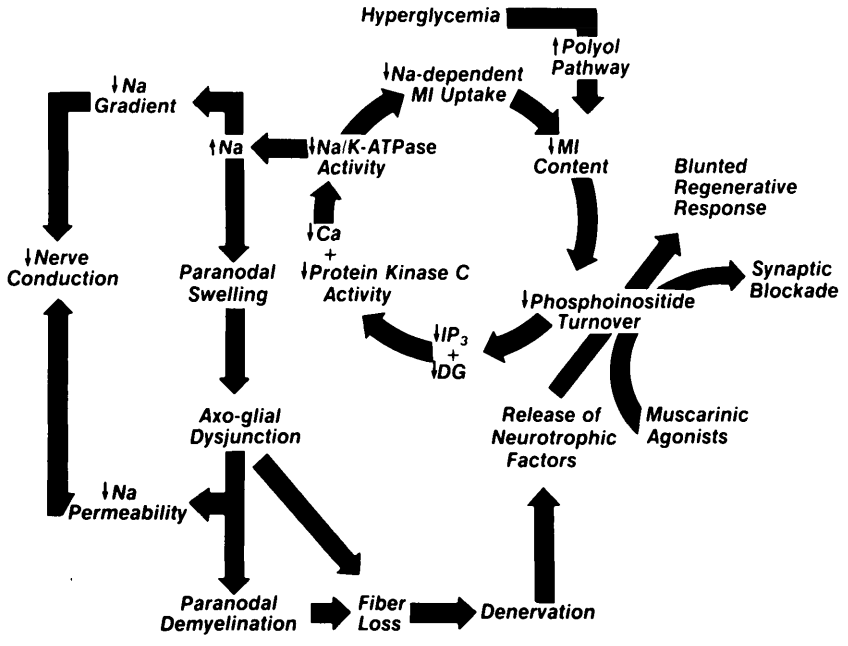

FIG. 1. Proposed pathogenetic scheme linking disturbances in nerve *myo*-inositol metabolism to slowing of nerve conduction; nerve fiber damage, demyelination, and degeneration; impaired synaptic transmission; and blunted nerve fiber repair and regeneration. Identity of neurotrophic factors is unknown but may include insulin-like growth factors and/or nerve growth factor. MI, *myo*-inositol.

potential importance of Na^+-K^+-ATPase in the generation and maintenance of the resting membrane potential of excitable cells focused several independent lines of investigation on a possible alteration in Na^+-K^+-ATPase function. Enzymatic measurements of Na^+-K^+-ATPase activity (25,34,36,62,63) and in vitro metabolic studies (53,64,65) indicated disturbed Na^+-K^+-ATPase function in diabetic nerve. Voltage-clamp studies of the node of Ranvier in the spontaneously diabetic BB rat implied the existence of a defect in the Na^+-K^+-ATPase function causing a fourfold increase in intracellular [Na^+] (24,66,67). The fact that *myo*-inositol replacement prevents or reverses impaired nerve conduction velocity in acute experimental diabetes implies that *myo*-inositol depletion must be responsible for the Na^+-K^+-ATPase defect if the latter is to explain nerve conduction slowing. Indeed, prevention or reversal of *myo*-inositol depletion in diabetic nerve by oral *myo*-inositol supplementation or administration of an aldose reductase inhibitor to rats with STZ-D or spontaneous diabetes normalizes enzymatically measured Na^+-K^+-ATPase activity in sciatic nerve (25,36,63). Because the major metabolic pathway for *myo*-inositol is reversible incorporation into phosphoinositides, a putative phosphoinositide link between *myo*-inositol deficiency and Na^+-K^+-ATPase regulation was sought. Phosphoinositide turnover yields several important classes of intracellular mediators, i.e., *sn*-1,2-diacylglycerols (DGs) and water-soluble inositol phosphates and their derivatives, which stimulate protein kinase C and translocate intracellular calcium, respectively (68). Modulation of Na^+-K^+-ATPase activity by either DG- or calcium-dependent protein kinases would explain the close empirical association between *myo*-inositol depletion and diminished Na^+-K^+-ATPase activity in diabetic nerve. Indeed, analogues of phosphoinositide-derived protein kinase C agonists rapidly reverse the Na^+-K^+-ATPase defect and its metabolic consequences in *myo*-inositol–depleted diabetic peripheral nerve (61,65) or isolated membranes derived therefrom (69) in vitro. The reduction in Na^+-K^+-ATPase activity in diabetic nerve probably mirrors decreased protein kinase C activity, which in turn reflects disturbed phosphoinositide metabolism that is secondary to depletion of *myo*-inositol in some component of peripheral nerve (Fig. 1, *top right*).

Decreased Na^+-K^+-ATPase activity probably plays a fundamental role in the acutely reversible conduction defect in diabetic rat nerve (34) and in the development of the earliest structural changes in experimental diabetic neuropathy. The reversible slowing of nerve conduction in the acutely diabetic BB rat parallels a fourfold rise in axonal Na^+ (24,66,67) associated with a marked swelling of the nodal and paranodal axon that is not attributable to sorbitol accumulation because it is reversed by dietary *myo*-inositol supplementation, which does not affect nerve sorbitol (24). Animals with more chronic diabetes develop a superimposed poorly reversible decrease in nodal Na^+ permeability and an increase in nodal K^+ permeability (67) associated with disruption of insulating junctional complexes between terminal loops of myelin and the paranodal axolemma (axoglial dysjunction; 70). Thus, corresponding and poorly reversible structural and functional changes at the node of Ranvier supervene at this chronic stage of experimental diabetes and account for that component of nerve-conduction slowing not readily reversed by metabolic correction (34,66,71). The basis of the axoglial dysjunction in the diabetic nerve has not been fully established, although it may represent persistent ultrastructural evidence of antecedent paranodal swelling (24). However, long-term treatment with an aldose reductase inhibitor reverses axoglial dysjunction in human diabetic neuropathy, implying that polyol-pathway–related defects in metabolism contribute to the persistence, if not the development, of this structural abnormality in humans (72; Fig. 1, *left*).

The pathogenesis of other structural changes, i.e., axonal atrophy and degeneration, is less well understood and may have many components, including nonenzymatic glycosylation of structural proteins, impaired axonal transport mechanisms, and impaired protein synthesis (73). However, relevant underlying functional abnormalities, i.e., impaired

axonal transport in experiments on animals with acute diabetes, are corrected by insulin therapy, *myo*-inositol supplementation, or aldose reductase inhibition (27,28,31,37, 74), suggesting that the same underlying basic metabolic abnormalities, including an altered phosphoinositide metabolism, may be at least partially responsible for the characteristic axonal atrophy and degeneration seen in diabetic neuropathy. Furthermore, as discussed below, a blunting of compensatory neurotrophism, which may be partially dependent on phosphoinositide-mediated signal transduction (75,76), could contribute to many potential components of axonal atrophy.

A corollary of the sorbitol–*myo*-inositol–Na^+-K^+-ATPase hypothesis for the pathogenesis of diabetic neuropathy is that interruption of this sequence with aldose reductase inhibitors or other forms of metabolic intervention should produce a detectable clinical response. However, short-term treatment with aldose reductase inhibitors improves nerve function in diabetic subjects but is associated with only marginal symptomatic improvement in patients with diabetic neuropathy (77). Because clinical responses may be delayed and difficult to detect with available tools of clinical assessment, the effect of an aldose reductase inhibitor, sorbinil, on the underlying biochemical abnormalities and neuropathologic lesions accompanying symptomatic diabetic peripheral polyneuropathy was explored in paired sural nerve fascicular biopsies obtained from 16 neuropathic subjects at entry and after completion of a 1-yr randomized, placebo-controlled, double-masked clinical trial of sorbinil (250 mg/day) (72). Sural nerve sorbitol levels declined, and *myo*-inositol levels, which were decreased at baseline, tended to improve in treated patients (78). Sorbinil therapy was also associated with microscopic evidence of enhanced nerve fiber regeneration and repair (72). The immediate clinical implications of this early fiber regeneration and repair are uncertain. This early stage of fiber repair and regeneration is more likely to herald than accompany any easily detectable clinical improvement, and it is not surprising that short-term clinical responses to aldose reductase inhibitors as documented in published trials would reveal only marginal clinical improvement at best. On the other hand, extrapolation of these early reparative- and regenerative-treatment responses to more prolonged administration of aldose reductase inhibitors would suggest ultimate significant reversal of the characteristic morphometric and perhaps clinical components of diabetic neuropathy in patients afflicted with the disorder. These possibilities provide a powerful rationale for the longer-term prospective clinical trials with aldose reductase inhibitors that are under way (Fig. 1, *bottom right*).

However, even if the entire contribution of hyperglycemia to the development of diabetic neuropathy were mediated by secondary abnormalities in sorbitol, *myo*-inositol, and phosphoinositide metabolism, other factors must also play a role. Note the differences in the histopathological picture of neuropathy in patients with IDDM and NIDDM despite similar durations and severity of diabetes (73), the apparent influence of age and gender on the appearance of early neuropathy in patients with IDDM (79), and the association of alcohol consumption with diabetic neuropathy (79,80). Although early metabolic and functional disturbances in diabetic nerve, i.e., impaired Na^+-K^+-ATPase function and paranodal swelling, are empirically attributable to abnormal *myo*-inositol and phosphoinositide metabolism, more advanced structural abnormalities may reflect superimposed independent biochemical and/or hormonal defects (although, as mentioned above, aldose reductase inhibition decreases axoglial dysjunction in diabetic humans). The peripheral nervous system has only a limited repertoire of responses to various insults; i.e., wallerian degeneration, axonal atrophy, impaired axonal transport, and dystrophic changes in diabetic neuropathy may be responses to multiple factors. However, the increasingly recognized importance of the phosphoinositide cascade in neuromodulation may attribute a progressively wider range of disturbances in the diabetic peripheral nervous system to *myo*-inositol depletion and associated defects in phosphoinositide metabolism. Thus, although all effects of aldose reductase inhibitors in the peripheral nervous system of diabetic rats have been reproduced by *myo*-inositol supplementation when this alternative intervention has been tested, the exact role of phosphoinositide metabolism in most of these responses is not entirely understood. For example, the ability of aldose reductase inhibitors to reduce the susceptibility of fast axonal transport to nerve compression in diabetic rats (81) might ascribe part of the vastly increased incidence of compression neuropathies in diabetic patients (82) to some as yet unknown alteration in phosphoinositide-related processes. In a more general sense, the recently recognized and still poorly understood role of phosphoinositide signal transduction in neurotrophism may ultimately explain why the impact of various insults to the peripheral nervous system appears to be magnified in diabetes. In fact, diminished regenerative compensation for the normal wear and tear of daily living on the peripheral nervous system may explain many of the degenerative aspects of diabetic peripheral neuropathy and why they are so nonspecific. Finally, synergistic effects of glucose-induced *myo*-inositol depletion and the hormonal disturbances of diabetes on neural phosphoinositide metabolism may greatly amplify the role of phosphoinositide-related defects in diabetic neuropathy. Therefore, although not by itself explaining the entire range of peripheral nerve disease in diabetic neuropathy, abnormal phosphoinositide metabolism may play a key role in uniting many of the disturbances of the peripheral nervous system produced by diabetes.

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