

Prospective Role of β -Cell-Specific IGF-1 for Oxidative Stress in the Pathogenesis of Diabetic Neuropathy

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Abstract

Diabetic neuropathy is a well-known complication of diabetes mellitus. The mechanism for progression of diabetic neuropathy is unclear, but many risk factors, such as abnormalities of glucose metabolism and oxidative stress, have been given much attention for their contributory role in the loss or degeneration of neurons. Homocysteine is a risk factor of cardiovascular disease and diabetes/metabolic disease. Homocysteine metabolism is dependent on vitamin B12 the deficiency of which induces peripheral neuropathy. On the other hand, the examination of generative pathology showed the potential involvement of many growth factors in neuroprotection and regeneration. This review implicates Insulin-like growth factor-1 (IGF-1) as playing a crucial role in pancreatic β -cell functions and homocysteine-induced oxidative stress. A defense mechanism against diabetic neuropathy via homocysteine-induced oxidative stress due to a protective effect of β -cell-specific IGF-1 will be discussed.

Keywords: Diabetic neuropathy; Homocysteine; IGF-1; Oxidative stress; β -cells; Glucose metabolism; HDL; LDL

Introduction

Neuropathy is the most common complication of diabetes, occurring in 60% of diabetic patients [1], and it causes significant morbidity and mortality. Over the past 30 years, diabetic neuropathy has been shown to involve many biochemical and functional abnormalities, in both diabetic patients and animal models [2]. While it is known that oxidative stress is related to the progression of nerve dysfunction and that growth factors are involved in beneficial protection against functional nerve failure, the mechanism by which the oxidative stress controls diabetic neuropathy has not yet been elucidated [3]. Considering the specific conditions under which peripheral neuropathy occurs in diabetes mellitus, we believe that pancreatic β -cell function plays a crucial role in neuroprotection [4]. Insulin-like growth factor-1 (IGF-1) is necessary both for β -cell function [5] and as a protective agent against neurodegeneration in diabetes [6].

In this review, I will explore the current knowledge of IGF-1 and homocysteine-induced oxidative stress, introduce our proposed mechanism of IGF-1-mediated neuroprotection against homocysteine-induced oxidative stress, and discuss the crucial role of β -cell-specific IGF-1 in diabetic neuropathy.

Homocysteine-Induced Oxidative Stress

Reactive Oxygen Species (ROS) are formed as natural toxic by-products of the normal metabolism of oxygen. Cells typically defend themselves against ROS damage using enzymes such as superoxide dismutases, catalases, lactoperoxidases, and glutathione peroxidases [7]. However, environmental stress, such as exposure to UV, ionizing radiation and heat, causes drastic increases in ROS levels [8]. This effect is known as oxidative stress. ROS interfere with the function of Nitric Oxide (NO), which is a key mediator of cell signaling and is critical to many important vascular and nervous functions [9].

Homocysteine which causes oxidative stress is known to enhance ROS levels in patients with vascular and neurodegenerative diseases [10]. Serum homocysteine levels are normally very low in healthy individuals (around 100 nM) [11], whereas elevated serum homocysteine levels cause remarkable ROS generation in endothelial cells, leading to

vascular injury [12]. In addition to the induction of ROS, homocysteine is connected to the oxidation defense system through the disulfide forms of homocysteine. Molecular targeting by homocysteine results in thiol-disulfide exchange reactions collectively called S-homocysteinylation, leading to the formation of stable covalent disulfide bonds with cysteine residues [13]. The non-protein-bound forms of serum homocysteine (free Hcy) account for 30% of total serum homocysteine; while protein-bound homocysteine (bound Hcy) accounts for 70% of total serum homocysteine (Figure 1). The sulfhydryl-reducing action of Cys³⁴-SH on serum albumin contributes to the defense against oxidative stress from ROS. In hyper-homocysteinemia, albumin appears as a carrier of disulfide-bonded homocysteine (Albumin-S-S-Hcy) [14,15]. This finding implies that a decrease of the reduced albumin (Albumin-SH) in circulation weakens the oxidation defense system. The cysteine molecule is only one methylene group shorter than homocysteine. Although serum cysteine levels are 25 times higher than that of homocysteine, and cysteine generates the disulfide form of albumin (Albumin-S-S-Cys), cysteine is not toxic and is not considered an oxidative risk factor. Homocysteine thiolate anion (Hcy-S⁻), however, is a very reactive nucleophile that undergoes thiol disulfide exchange with Albumin-S-S-Cys to form Albumin-S-S-Hcy. This chemical mechanism is driven by a difference in dissociation constant (pKa) values: cysteine thiolate anion, with pKa = 8.3; and homocysteine thiolate anion, with pKa = 10.0. These values are reflective of the fact that Cys-S⁻ is a preferable leaving group because of its greater stability than Hcy-S⁻ [16]. An excess amount of serum homocysteine generates Hcy-Thiolactone (HT), which is formed by cyclic thioesterification of homocysteine, as in methionyl-tRNA (Met-tRNA) synthesis (Figure 2). HT is toxic, as it leads to N-homocysteinylolation (homocystamide formation) of sev-

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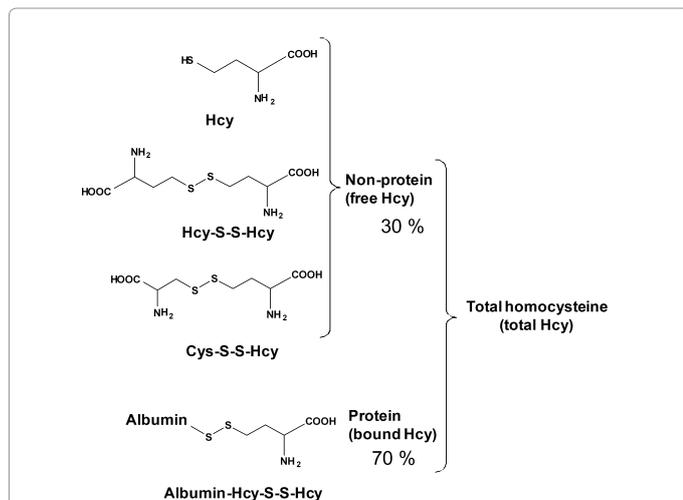


Figure 1: Influence of homocysteine-induced products on the balance between oxidation and reduction states

Homocysteine (Hcy) and its oxidized products are seen in normal human serum. Albumin with Cys³⁴-SH (Albumin-SH) contributes to anti-oxidative stress reactions and is protective against Reactive oxygen species (ROS). This disulfide adducts of homocysteine control the whole body- balance between oxidation and reduction.

inactivation, protein aggregation and eventual precipitation[16]. In particular, N-homocysteinylated of Low-Density Lipoprotein (LDL) is suggested to exert oxidative stress, leading to cardiovascular damage in type 1 diabetes [17]. Paoli P et al. [18] reported that protein N-homocysteinylated induces the formation of toxic amyloid-like protofibrils.

Homocysteine-Thiolactonase

Homocysteine is produced by the intracellular demethylation of methionine, which then enters the transsulfuration pathway or the remethylation cycle, as shown in Figure 2. Fifty percent of homocysteine enters the transsulfuration pathway, where it is irreversibly combined with serine to form cystathionine via the B6-dependent enzyme, Cystathionine β -Synthase (CBS) [15,19]. This cystathionine is then metabolized to cysteine via another B6-dependent enzyme cystathionine γ -lyase (CSE) and ultimately to sulfate, which is excreted in the urine. The other 50% of homocysteine enters the remethylation pathway and is recycled to methionine. Homocysteine is converted back to methionine by two different reactions, either catalyzed by Betaine-Hcy Methyltransferase (BHMT) or Methionine Synthase (MS), the latter reaction requiring 5-methyltetrahydrofolate as methyl donor and vitamin B12 as a cofactor. Thus, these two pathways for intracellular homocysteine metabolism can be impaired either by genetic defects in the enzymes needed for homocysteine metabolism or by the nutritional deficiency of the necessary vitamin cofactors such as folate, B12, and B6 [15]. Such metabolic deterioration of the remethylation cycle and transsulfuration pathway induces formation of either disulfide adduct or Hcy-thiolactone [20]. Hcy-thiolactone then causes protein N-homocysteinylated and subsequent protein damage [21]. In particular, homocysteinylated LDL becomes more susceptible to lipid peroxidation [22]. On the other hand, High-Density Lipoprotein (HDL) is resistant to N-homocysteinylated because of an associated Hcy-thiolactonase (HTase), hydrolyzing enzyme of Hcy-thiolactone; this hydrolysis prevents subsequent HDL peroxidation. Hcy-thiolactone is reported to produce oxidative stress in rat hippocampal neurons by inhibiting Na⁺/K⁺-ATPase activity [23,24]. Further, Vignini et al. [25] reported that HT-modified LDL (HT-LDL) attenuates Na⁺/K⁺-ATPase activity in cultured human aortic endothelial cells.

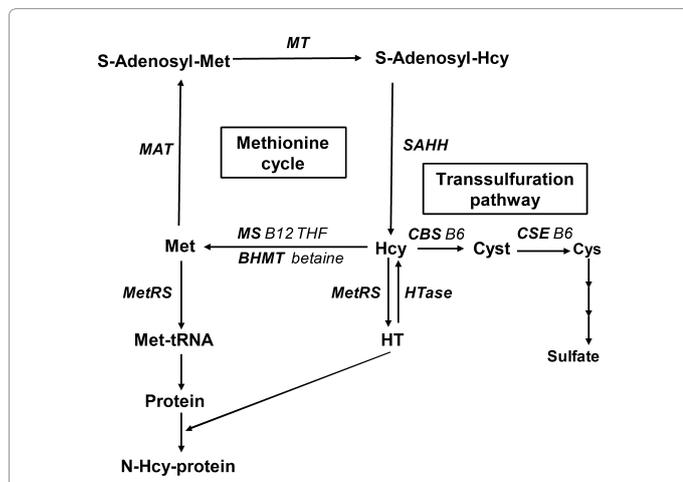


Figure 2: Homocysteine metabolism

Normally, homocysteine (Hcy) is maintained at very low levels in serum due to the methionine cycle, which leads to a rapid methyl transfer reaction using the following enzyme substrates: methionine (Met), S-adenosyl-methionine (S-Adenosyl-Met), and S-adenosyl-homocysteine (S-Adenosyl-Hcy). The methionine cycle is controlled by methionine synthase (MS), betaine-Hcy methyltransferase (BHMT), methionine adenosyltransferase (MAT), methyltransferase (MT), and S-Adenosyl-Hcy hydrolase (SAHH). Met and Hcy are also metabolized by methionyl-tRNA synthase (MetRS) to become Met-tRNA and Hcy-thiolactone (HT). Under normal conditions, Hcy-thiolactone is rapidly converted to Hcy by Hcy-thiolactonase (HTase). However, Hcy-thiolactone reacts spontaneously with protein ϵ -lysine residues, leading to formation of N-Hcy-protein. Alternatively, Hcy can enter the transsulfuration pathway. It is then converted to cystathionine (Cyst) by cystathionine β -synthase (CBS), which is further converted to cysteine (Cys) by cystathionine γ -lyase (CSE). The transsulfuration pathway ends with sulfate. Additionally, vitamins, including: B6, B12, betaine (B10), and tetrahydrofolate (THF, sometimes called B9).

Hcy-thiolactone is thought to be an endogenous substrate for the serum arylesterases/paraoxonase-1 (PON1) [26]. PON1 has been implicated in the detoxification of various organophosphatases, such as nerve gases, dietary neurotoxins, or toxic lipids produced during oxidative stress [27]. Serum PON1 activity is decreased in type 2 diabetic patients with atherosclerosis [28] as well as in type 1 diabetic rats [29]. Decreased PON1 activity is also observed in patients with type 1 and type 2 diabetic peripheral neuropathy, suggesting that diabetic neuropathy may also arise, in part, due to the increased susceptibility of the nervous system to neurotoxic damage resulting from the diabetic's lack of protection by PON1 [30]. The influence of PON1 activity on HDL in diabetes has become another important concern [31,32]. We believe HTase activity can be an important biomarker for evaluating diabetic neuropathy, especially when estimation of this enzyme activity requires Hcy-thiolactone to be a natural substrate.

Homocysteine and LDL/HDL Cholesterol Transportation

Cholesterol synthesis occurs mainly in the liver, although the Central Nervous System (CNS) synthesizes its own considerable supply [33]. Other extra hepatic organs synthesize cholesterol *de novo* after uptake of the necessary components from circulating LDL, with cholesterol esterification occurring in the liver. The flow of cholesterol me-

eral serum proteins, including N-Hcy-hemoglobin (75%) and N-Hcy-albumin (22%). N-homocysteinylated of these proteins causes enzyme

metabolism in the Peripheral Nervous System (PNS) is not well understood, but it seems to involve a homeostatic mechanism for regulating cholesterol, similar to that demonstrated in the CNS. Turnover of PNS cholesterol is also regulated by this balanced system whereby the liver coordinates cholesterol synthesis and metabolism with all other organs via LDL-cholesterol and HDL-cholesterol in the blood circulation (Figure 3). LDL cholesterol is transported across the Blood Nerve Barrier (BNB) into PNS neurons via a LDL-receptor. LDL can homocysteinylate its proteins via Hcy-thiolactone to become oxidized LDL, which is a toxic substance causing a great deal of oxidative stress [34]. HTase, found in HDL, is an enzyme that protects against Cardiovascular Disease (CVD) by detoxifying Hcy-thiolactone and preventing the formation of oxidized LDL [31]. As illustrated in Figure 3, our hypothesis regarding diabetic neuropathy is based on the homocysteamide-related mechanism for elevating serum homocysteine.

IGF-1 and Oxidative Stress

Studies on the pathology of diabetic neuropathy have encouraged further investigation of the influence of various growth factors on PNS-degenerative processes such as demyelination and axonal injury. In particular, there has been extensive research on the role of neurotrophins, insulin-like growth factors, Ciliary Neurotrophic Factor (CNTF), and Glia-Derived Neurotrophic Factor (GDNF) [35].

Serum IGF-1 is known to be decreased in rats and humans with

diabetic neuropathy [36,37]. The observation of low serum IGF-1 levels in human patients and animal models of different types of neurodegenerative diseases led to therapeutic use of IGF-1 to restore normal serum levels [38]. In addition, IGF-1 is a glucose-dependent growth factor and is closely associated with diabetes mellitus. Glucose is implicated as a regulatory molecule for inducing β -cells to secrete insulin and IGF-1. It is known that this glucose-dependent IGF-1 activation system is closely coupled to glucose metabolism via such mechanisms as the glycolytic pathway and the pentose phosphate pathway [5,39,40]. For example, activation of the glucose-dependent IGF-1 system subsequently enhances the glycolytic pathway for cell proliferation [5]. There are also well-known specific inhibitors for each of these pathways: 6-aminonicotinamide (6-AN) for the pentose phosphate pathway, and 2-deoxy-glucose (2-DG) for the glycolytic pathway [41,42].

Islet β -cell dysfunction also occurs from the oxidative stress of elevated ROS levels, which are misregulated by the defense system in diabetic islet β -cells suffering diabetes mellitus. Streptozotocin (STZ) is a toxic chemical that induces type 1 diabetes mellitus when injected into rats; these STZ-diabetic rats have provided a useful etiological model for studying diabetic neuropathy caused by oxidative stress. It has been suggested that STZ may generate ROS such as NO and O_2^- , thereby inducing apoptosis of pancreatic β -cells [43-45]. It is reported that homo-

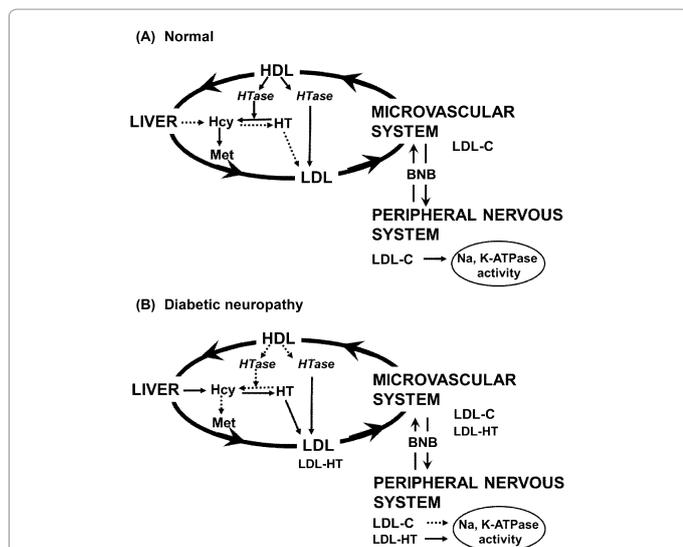


Figure 3: Effect of homocysteine on the HDL/LDL cholesterol transport and the peripheral nervous system

Under normal conditions, most, but not all, of homocysteine (Hcy) is converted into methionine. Excessive amounts of Hcy-thiolactone (HT) are eliminated from the circulation immediately, and are metabolized by HTase, which detoxifies the HT.

Under diabetic conditions, however, excessive Hcy is produced and converted to HT, which appears in the circulation. Decreasing HDL-cholesterol (HDL-C) levels reflect an equivalent lessening of the HDL-related enzyme, HTase. The decreased amount of HTase allows LDL proteins to undergo N-homocysteinylate. HT-modified LDL in circulation attacks the microvasculature and invades the peripheral nervous system through the blood nerve barrier (BNB). Eventually, the circulating HT-modified LDL shows neurotoxicity through suppression of Na, K-ATPase activity in myelin-making Schwann cells. Thus diabetic neuropathy develops with a reduction of normal membrane potential. Keen arrows indicate direction of flow of cholesterol transport system. Line arrows indicate stimulation of enzyme activity, metabolite production, or oxidized modification. Dotted line arrows indicate suppressions of enzyme activity, metabolite production, or oxidized modification.

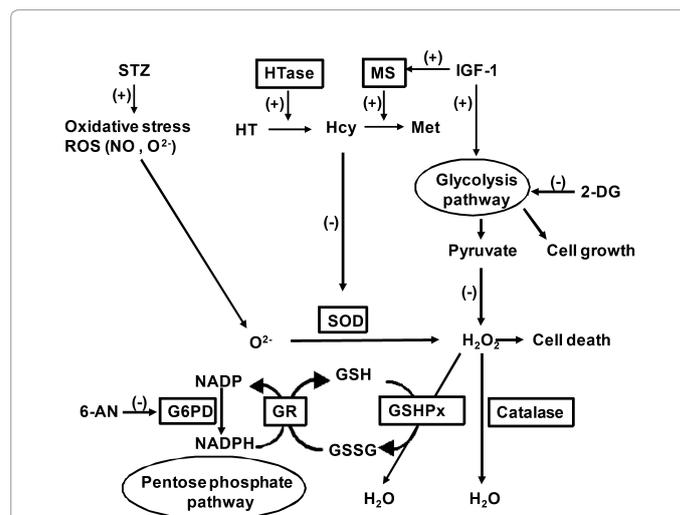


Figure 4: Protective mechanism of β -cell-specific IGF-1 against oxidative stress in pancreatic β -cells

Reactive oxygen species (ROS) generated from STZ exposure are metabolized and inactivated by superoxide dismutase (SOD) to produce H_2O_2 . An imbalance in the coordinated expression/activity of glutathione peroxidase (GSHPx) and glutathione reductase (GR) can cause excessive generation of ROS, leading to oxidative stress. GSHPx converts H_2O_2 to water using glutathione (GSH) and produces oxidized glutathione (GSSG). Cellular maintenance of a balanced redox state is controlled by intracellular regulators such as reduced GSH and nicotinamide adenine dinucleotide phosphate (NADPH). Both GR and glucose-6-phosphate dehydrogenase (G6PD) are enzymes with expected protective activity against oxidative stress. 2-deoxy-glucose (2-DG, an inhibitor of the glycolytic pathway) and 6-aminonicotinamide (6-AN, an inhibitor of the pentose phosphate pathway) both decrease cellular levels of pyruvate and NADPH. This leads to an accumulation of H_2O_2 , the buildup of which induces apoptosis. The protective action of β -cell-specific IGF-1 is exerted via an increase in two targets: increasing methionine synthase activity (MS), which enhances homocysteine metabolism (Figure 2); and enhancing the glycolytic pathway, leading to cellular elevation of pyruvate, which inhibits of H_2O_2 -induced cell death. Enhancement of the glycolytic pathway also leads to an intracellular environment favorable to cell growth. Arrows with (+) and (-) respectively represent stimulation and suppression.

cysteine also impairs β -cell functions such as insulin secretion through alterations in β -cell glucose metabolism [46,47].

Figure 4 illustrates the primary defense mechanism against oxidative stress in β -cells. β -cells bind and take up LDL via cell surface receptors [48]. Oxidized LDL can damage the β -cells [49]. It is reported that this detrimental effect is detoxified by PON1/HTase, suggesting the intrinsic presence of HTase in β -cells, in addition to the presence of HDL-associated HTase [50]. As shown in Figure 4, the proposed mechanism for defense against oxidative stress in β -cells is controlled by Superoxide Dismutase (SOD), Glutathione Peroxidase (GSHPx), and catalase, all of which act to control the level of ROS produced during oxidative stress. Maintenance of the redox status in cells is performed by intracellular regulators, reduced glutathione (GSH), and NADPH. This mechanism involves two enzymes: Glutathione Reductase (GR) and Glucose-6-Phosphate Dehydrogenase (G6PD). The overall effect of the antioxidant system is always to maintain the intracellular balance between these antioxidant enzymes [51], as a critical balance exists in the β -cells between endogenous ROS generation and antioxidant defense.

Additionally, IGF-1 can affect normal cellular differentiation and dedifferentiation via DNA methylation. Methylation reactions, including DNA methylation and homocysteine methylation, are controlled by methionine synthase (MS) in the methionine cycle (Figure 2). Therefore, decreased methylation of homocysteine also implies deficient DNA methylation, raising the coincidence of interfering with IGF-1 responses. It is reported that IGF-1 functions by altering MS activity [52,53]. Up-regulation of IGF-1 increases MS activity, which is connected to amelioration of associated Hcy-oxidative stress.

β -Cell-Specific IGF-1 and Diabetic Neuropathy

IGF-1 is expressed in various tissues including brain, bone, muscle, and liver, but approximately 75% of IGF-1 is expressed in the liver [54]. Liver-specific IGF-1 knockout (KO) mice showed reduced body weights and increased life spans [55]. However, these findings did not suggest something remarkable in neuronal function to be supported by liver-specific IGF-1. Thus, it has remained unknown whether the beneficial effects of liver-derived IGF-1 extend to protection of the PNS in animals with diabetic neuropathy. Because observable pathology, such as axonal atrophy or neurofilament loss, occurs much later than does the slowing of nerve conduction velocity in peripheral nerves of diabetes [4], abnormal glucose metabolism together with oxidative stress may be importantly related to the early stages of onset of diabetic neuropathy. After partial pancreatectomy in the dog, pancreatic tissue is regenerated by remarkably enhanced expression of pancreas-specific IGF-1 [56]. It is reported that both IGF-1 and its receptor are expressed in pancreatic islet cells [57,58]. Furthermore, autocrine expression of β -cell-specific IGF-1 has been demonstrated to prevent STZ-induced oxidative stress and β -cell apoptosis [59]. Rather than the originally proposed liver-specific IGF-1 or other tissue-specific IGF-1, we now believe that β -cell-specific IGF-1 may be closely connected to an intrinsic action in pancreas to resist damage from oxidative stress and prevent eventual peripheral nerve degeneration.

It has been reported that methylcobalamin is useful for treatment of diabetic neuropathy, based on an observation that the up-regulation of peripheral nerve IGF-1 gene expression occurs after intramuscular injection of methylcobalamin [60]. Clinical studies with L-methylfolate and methylcobalamin showed restoration of loss of skin sensation in patients with diabetic neuropathy [61]. These B vitamins are involved in homocysteine metabolism and may be connected to a defense mechanism against homocysteine-induced oxidative stress.

β -Cells and Diabetic Autonomic Neuropathy

Histological studies have demonstrated the presence of both cholinergic and adrenergic nerve fibers in the pancreas. Pancreatic islet innervations include peptidergic, cholinergic, adrenergic, and GABAergic fibers [62]. Acetylcholine the major parasympathetic neurotransmitter, is released by intrapancreatic nerve endings during the pre-absorptive and absorptive phase of feeding [63]. The overall effect of this parasympathetic stimulation of β -cells is increased insulin secretion [64]. On the other hand, the sympathetic innervation of β -cells provides release of norepinephrine. Catecholamines such as epinephrine and norepinephrine are known to inhibit insulin secretion in vivo and in vitro [65]. Exocrine pancreatic insufficiency in diabetes mellitus has been attributed to diabetic neuropathy [66]. Alterations in various sympathetic autonomic ganglia were observed in autonomic neuropathy of STZ-induced diabetic rats [67]. In comparison, relatively little is known concerning the effect of β -cell-specific IGF-1 on autonomic diabetic neuropathy. Based on the known mechanism for autonomic dysfunctions in islet cells, we suggest that β -cell-specific IGF-1 may be a promising growth factor for treatment of autonomic dysfunction together with diabetic neuropathy.

Conclusion

Homocysteine is known to be a risk factor for diabetic neuropathy; specifically, homocysteine-induced oxidative stress is involved in the development of diabetic disease. IGF-1, on the other hand, plays an important role in protection of peripheral neurons against oxidative stress and amelioration of neuronal degeneration. Since pancreatic β -cells are associated with the homocysteine-induced oxidative stress of diabetes, there is a possibility that β -cell function is involved in a progression of diabetic neuropathy. As a consequence of these connections, I propose that β -cell-specific IGF-1 activates a defense mechanism against homocysteine-induced oxidative stress.

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