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Diabetic Autonomic Neuropathy

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Abstract

It has been estimated that up to 50% of patients with type 1 or type 2 diabetes will have the complication of neuropathy. The impact of autonomic neuropathy in diabetes is often underestimated, but it can result in a range of debilitating symptoms including orthostatic hypotension, gastroparesis, disordered gastrointestinal motility, impotence and bladder dysfunction, and it also carries an increased risk of cardiovascular mortality. Reduced blood supply, impaired nerve regeneration, deficient axonal transport, loss of neurotrophic support and metabolic changes induced by hyperglycemia including mitochondrial dysfunction have all been implicated in the development of diabetic neuropathy. Many of these deficits can lead to the production of oxidative stress that appears to be a common factor leading to nerve damage. Studies of animal models have revealed that not all subpopulations of autonomic nerves degenerate in diabetes and that some populations are more difficult to treat once neuropathy has developed. Recent evidence indicates that differences in the level of metabolic activity and in the intrinsic defence mechanisms of subpopulations of autonomic neurons may account for their differential susceptibility to the development of neuropathy in diabetes. (*Tzu Chi Med J* 2008;20(3):161–168)

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1. Introduction

The incidence of diabetes mellitus has been described as reaching epidemic proportions, particularly in the Western world. It has been estimated that up to 50% of patients with type 1 or type 2 diabetes will have the complication of neuropathy (1). When the autonomic nervous system is affected, this can lead to a variety of symptoms such as tachycardia, orthostatic hypotension, gastroparesis, constipation, diarrhea, fecal incontinence, impotence and bladder dysfunction, thus significantly affecting the quality of life of diabetic patients. Furthermore, the presence of autonomic neuropathy carries a significant increased risk of cardiovascular mortality. Deficits in the autonomic supply to the skin can also disrupt microvascular flow and impair sweating, contributing to the development of foot ulcers that occur as a consequence of the sensory deficits associated with sensory neuropathy in diabetes (2,3). Chronic foot ulcers that fail to heal are a major cause of nontraumatic amputation. There is thus a substantial clinical need to understand the mechanisms underlying autonomic neuropathy in diabetes and to find potential treatments that can

prevent its development and treat the condition once it has occurred. Despite this, it has been observed that autonomic neuropathy is the least recognized and least understood complication of diabetes [3].

2. Clinical assessment

Historically, the assessment of peripheral neuropathy in diabetes has focused on myelinated nerves, largely due to technical reasons. In morphometric studies of human sural nerve biopsies, the standard tissue for microscopy analysis, it has proved easier to quantify changes in myelinated axons than unmyelinated axons. Similarly, the standard functional test of nerve conduction velocity provides no information on conduction in unmyelinated nerves [1,4,5]. With the introduction of new immunohistochemical markers and microscopy techniques, the use of skin biopsies in the detection of diabetic neuropathy has recently been developed. A loss of small unmyelinated sensory fibers has been demonstrated in the skin from diabetic patients. Significantly, a loss of small sensory fibers has been observed in diabetic patients with normal conduction velocity measurements [1,5]. This coincides with the view that changes in unmyelinated nerve fibers (both small sensory and autonomic) occur at an early stage in diabetes [4]. Whether a clinical entity separate from peripheral neuropathy exists in diabetes in which there is selective loss of small fibers with relative preservation of large sensory fibers, termed "small fiber neuropathy" is still a matter of debate [6,7].

One of the problems with evaluating the particular significance of autonomic neuropathy in diabetes is that pathological changes in autonomic nerves can be present before the development of overt symptoms. Furthermore, autonomic function is difficult to assess clinically using noninvasive techniques. At present, the only standard approach used to evaluate autonomic function in humans with diabetes relies on the measurement of heart rate and blood pressure responses to deep breathing, standing and the Valsalva maneuver. Loss of heart rate variability is the earliest indicator of cardiovascular autonomic neuropathy, and 7–10% of newly diagnosed type 1 diabetes patients have been reported to have abnormal heart rate variability. Thus, there is evidence, at least for within the cardiovascular system, that autonomic neuropathy is an early complication of diabetes (see reference 8 for review). Furthermore, the 5-year mortality rate in diabetic patients with evidence of cardiovascular autonomic neuropathy is significantly higher than in diabetic patients without such signs [3,8,9]. Research is ongoing into the development of alternative tests of autonomic function including assessment of pupillary, sudomotor, bladder and erectile function (see reference 3 for review). However, these have yet to become routine clinical practice. Furthermore, the majority of such tests assess reflex activity and do not provide information on the specific sites within peripheral autonomic pathways that are most susceptible to damage. The presence of pathological changes in the autonomic supply of visceral organs in diabetes in humans is often inferred from indirect evidence such as the presence of abnormal cardiovascular function tests and/or overt symptoms. Therefore, elucidation of the nature of the pathological changes that occur in autonomic nerves in diabetes has largely relied on studies of animal models.

3. Autonomic neuropathy in experimental diabetes

There are two main categories of animal models of diabetes: (1) those in which type 1 diabetes is induced by a chemical toxin that destroys the insulin-secreting pancreatic islet cells; and (2) those in which strains of mice or rats that are genetically predisposed to developing either type 1 or type 2 diabetes are selectively bred. The most commonly used chemical model is the streptozotocin (STZ)-diabetic rat. More recently, two spontaneously diabetic rat strains (BB/Wor and BBZDR/Wor), closely related genetically, are receiving considerable attention. These strains enable the comparison of neuropathic changes in type 1 and type 2 diabetes since the BB/Wor rat is a hyperglycemic, hypoinsulinemic model of type 1 diabetes and the BBZDR/Wor rat is a hyperglycemic, hyperinsulinemic model of type 2 diabetes [10]. A detailed description of all the changes that have been observed in the autonomic nervous system in experimental diabetes is beyond the scope of this brief review (see reference 11 for a comprehensive overview). Therefore, only characteristic or significant features will be highlighted here.

Where comparable tissues have been available for study, the results are encouraging that changes that occur experimentally in animals also occur in diabetes in humans. For example, impaired parasympathetic control of erectile tissue mediated by nitric oxide has been implicated in erectile dysfunction in both clinical and experimental diabetes [12,13]. Similarly, the presence of neuroaxonal dystrophy in sympathetic celiac/superior mesenteric ganglia (CG/SMG) has been observed in specimens from humans with diabetes and from rat and mouse models of type 1 diabetes [10,11,14].

Microscopy studies at the light and ultrastructural level have revealed evidence for degenerative changes in autonomic nerves in experimental diabetes. With respect to the sympathetic supply to the small intestine, dystrophic changes have been reported both within the CG/SMG, where the neuronal cell bodies are located, and in their axons supplying the ileum [11].

Terminal tyrosine hydroxylase (TH)-containing nerve fibers within the target have swollen varicosities, a feature characteristic of a degenerative process [15]. Noradrenaline (NA) and its synthetic enzyme, TH, accumulate within the cell bodies of the CG/SMG possibly due to a defect in axonal transport, whereas both NA and TH levels are reduced in the target ileum [11,16]. A similar pattern has been observed for the intrinsic neurons containing vasoactive intestinal polypeptide (VIP) located in the myenteric plexus that innervate the smooth muscle of the ileum. VIP accumulates in the myenteric neuronal cell bodies, VIP-containing nerves fibers within the smooth musculature have swollen varicosities with ultrastructural evidence of degeneration and fail to release VIP on nerve stimulation [15,17,18]. These features reflect the dying back type of distal axonopathy that is generally held to be the characteristic feature of diabetic autonomic neuropathy [11].

Whether neuronal cell death and loss of autonomic neurons occur in diabetes remain a matter of controversy. The issue of neuronal cell death was raised when it was reported that both high glucose *in vitro* and STZ-diabetes *in vivo* caused apoptosis in sensory neurons located in the lumbar dorsal root ganglion (DRG), and it was suggested that this contributes to the development of axonal degeneration [19]. Since this report, other studies of DRG in diabetes have found conflicting results (see reference 20 for review). It has been proposed that diabetes may cause activation of caspases but that this may not progress fully down the apoptotic pathway to cause cell death [21]. In the sympathetic SMG, neuronal cell counts have not provided any evidence of neuronal loss in diabetes at a stage when degenerative changes in axons can readily be observed [22]. This implies that neuronal cell death is not a prerequisite for axonopathy to occur. However, apoptosis has been reported in the pelvic ganglion neurons containing neuronal nitric oxide synthase (nNOS) that supply erectile tissue in STZ-diabetic rats [13].

One important feature of autonomic neuropathy revealed by studies of experimental diabetes is that not all populations of autonomic nerves are affected in diabetes. Thus, neuroaxonal dystrophy and accumulation of TH occur in the prevertebral sympathetic ganglion, the CG/SMG, but not in the paravertebral sympathetic superior cervical ganglion (SCG) from the same animals. Dystrophic changes occur in sympathetic axons supplying the ileum, located in the mesentery, but not in the sympathetic axons supplying the mesenteric blood vessels [11]. Similarly, intrinsic myenteric neurons containing VIP undergo degenerative changes in diabetes whereas intrinsic myenteric neurons containing substance P or neuropeptide Y appear to be relatively unaffected [17,23]. In the pelvic ganglion, apoptosis has been reported to occur selectively in nNOS-containing parasympathetic neurons in STZ-diabetic rats with sympathetic pelvic ganglion neurons containing TH being spared [13]. Why some autonomic nerves are more vulnerable in diabetes than others is an interesting question and answering it could shed some light on the mechanisms underlying the development of autonomic neuropathy.

4. The pathogenesis of neuropathy

A number of theories have been proposed to account for the development of neuropathy in diabetes, including: diabetes-induced deficits in the blood supply to nerves; metabolic changes induced by hyperglycemia; impaired nerve regeneration following a continuous insult; Schwann cell abnormalities; and deficient neurotrophic support [4,20,24–27]. Since the *Diabetes Control and Complications* study [28] reported that the major factor that correlated with the presence of neuropathy in diabetic patients was the degree of glycemic control, research has focused on the metabolic changes that can be induced by high levels of glucose. However, this does not preclude the involvement of the vascular supply and trophic support in neuropathy. Glucose-induced changes can not only affect axons directly but can also act by inducing changes either in the vascular system thus causing microangiopathy and reduced nerve blood flow or can affect Schwann cells thus impairing trophic support. Furthermore, neurotrophic factors may play a significant role in protecting the peripheral nervous system against the adverse consequences of glucose-induced metabolic changes and in improving nerve regeneration in diabetes.

4.1. Oxidative stress induced by high glucose levels

Glucose enters neurons via facilitated concentration-dependent transport. Therefore, hyperglycemia in diabetes results in increased intracellular concentrations of glucose that can lead to a number of metabolic changes, many of which cause oxidative stress. This has been reviewed extensively (see references 20, 24, 29). Oxidative stress occurs when there is a shift in the balance between the production of reactive oxygen species (ROS) and the antioxidant defence systems within a cell in favor of ROS production. Under normal conditions, the major site for formation of ROS is within mitochondria and this occurs as a by-product of oxidative phosphorylation. Neurons have a high level of metabolic activity and relatively high numbers of mitochondria. In common with other cells, they also have a variety of mechanisms to defend themselves against ROS. Within mitochondria, the two main

protective mechanisms are manganese superoxide dismutase (MnSOD) and glutathione peroxidase (GPx4). MnSOD converts superoxide anions to hydrogen peroxide while GPx4 converts hydrogen peroxide to water oxidizing glutathione in the process. Glutathione is a major intracellular antioxidant and has to be continually recycled from its oxidized form by the action of the enzyme glutathione reductase to maintain its antioxidant capacity. In the cytoplasm, alternative forms of the antioxidant enzymes (Cu/ZnSOD and GPx1) are present and catalyze similar reactions. Additionally, catalase converts hydrogen peroxide to water. Vitamin C, vitamin E and taurine also all have antioxidant properties. Vitamin C acts in the aqueous environment of the cell whereas vitamin E acts within the hydrophobic environment of membranes. Taurine is not only an antioxidant but is also released from cells under conditions of osmotic stress to maintain the internal osmolarity of the cell.

Several metabolic changes have been proposed to occur as a consequence of high intracellular levels of glucose leading to oxidative stress [20,24,29] (Fig. 1). The enzyme aldose reductase is activated to convert glucose to sorbitol during which process NADPH is oxidized to $NADP^+$. Subsequently, sorbitol dehydrogenase converts sorbitol to fructose. Thus, increased aldose reductase activity has the potential to cause depletion of NADPH. One of the consequences of NADPH depletion is impaired recycling of the antioxidant glutathione by glutathione reductase since this is also an NADPH-dependent enzyme. Increased intracellular glucose and fructose also cause osmotic stress stimulating the release and thus intracellular depletion of the antioxidant taurine. Glucose itself can undergo autoxidation, forming reactive species. Under normal conditions, glycation of macromolecules only occurs by an enzymatic process. However, at high concentrations, both glucose and fructose can glycate macromolecules via a nonenzymatic process. Following a series of chemical modifications, this results in the formation of advanced glycation endproducts (AGEs) that are not only highly reactive but can also act on the AGE receptor (RAGE), inducing oxidative stress.

Oxidative stress can lead to mitochondrial dysfunction, resulting in a vicious cycle in which increased mitochondrial production of ROS is induced following the production of ROS in the cytoplasm. However, a more direct pathway linking high glucose to increased ROS production by mitochondria has recently been proposed. High glucose levels can lead to high levels of NADH via the tricarboxylic acid cycle. Subsequently, excessive flux of NADH through the electron transport chain can lead to increased production of ROS by mitochondria. There is also preliminary evidence that diabetes can result in reduced expression of uncoupling protein-3. Uncoupling proteins dissipate the proton electrochemical gradient that is formed during respiration. Therefore, a reduction in uncoupling capacity will impair the ability of mitochondria to dissipate the proton electrochemical gradient, favoring the production of ROS [30]. It should be emphasized that the majority of the evidence for the metabolic changes described here has been obtained from *in vitro* studies of sensory neurons and it is generally assumed that similar mechanisms occur *in vivo* and also account for autonomic neuropathy.

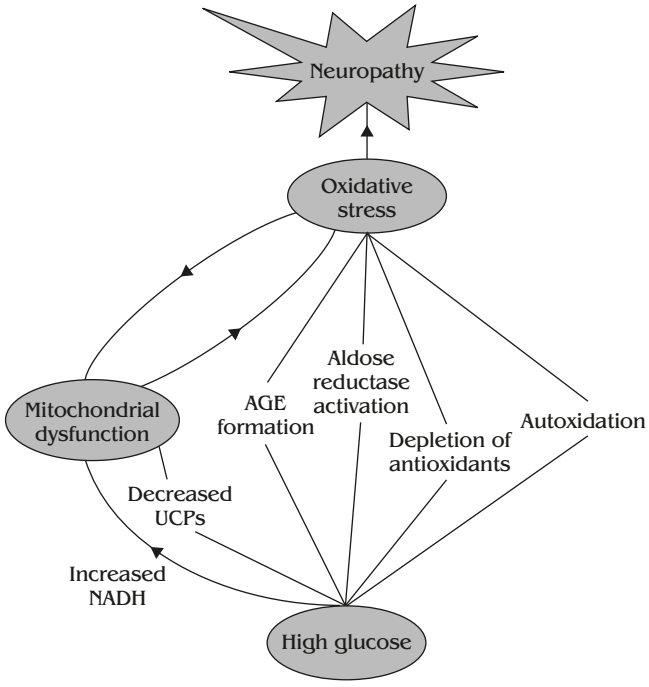

Fig. 1 — Schematic representation of the pathways that have been proposed to link the presence of hyperglycemia in diabetes with the production of oxidative stress and the development of neuropathy. High intracellular levels of glucose can: undergo autoxidation; lead to the formation of advanced glycation endproducts (AGEs); and activate aldose reductase which, together with osmotic stress, can cause depletion of antioxidants. The presence of oxidative stress can lead to mitochondrial dysfunction resulting in a vicious cycle where the mitochondria produce more reactive oxygen species (ROS) in response. High glucose may also cause increased flux of NADH through the electron transport chain and may decrease expression of uncoupling proteins (UCPs), both of which favor increased mitochondrial ROS production leading to oxidative stress.

4.2. Deficits in trophic factors

All the neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4, have been suggested to a greater or lesser extent to have a protective role in diabetes, and loss of target-derived neurotrophins in diabetes

has been implicated in the development of diabetic sensory neuropathy (see reference 25 for review). NGF has received the greatest attention since it has the capacity to act on both small DRG neurons with nociceptive function and on autonomic sympathetic neurons. *In vitro*, NGF can protect against oxidative stress and induce the expression of antioxidant defence enzymes (31,32). NGF treatment of diabetic rats has been shown to prevent and/or reverse diabetes-induced deficits in sensory neurons (33–35), although clinical trials have proved less successful (36). In contrast, NGF treatment of rats had no beneficial effect on neuroaxonal dystrophy induced by diabetes in the sympathetic SMG neurons. Furthermore, treatment of control rats caused neuroaxonal dystrophy in the SMG (37). Thus, it cannot be assumed that treatments for diabetic neuropathy will be equally successful for all subpopulations of peripheral neurons.

More recently, attention has been paid to the neurotrophic effects of the family of growth factors consisting of insulin-like growth factor 1 (IGF1), insulin and C peptide. IGF1 resembles proinsulin, the precursor that is cleaved to produce insulin and C peptide. In type 1 diabetes, there are reduced circulating levels of insulin, C peptide and IGF1 (25,38). There is *in vitro* evidence that all three factors can increase neuronal survival and neurite outgrowth (39–41). In addition, supplementation of diabetic rats with IGF1, insulin or C peptide has been shown to prevent the development of sensory deficits in rats *in vivo* even when hyperglycemia is still present (38,42,43). Furthermore, IGF1 reverses sympathetic neuroaxonal dystrophy in diabetic rats (44). The significance of these findings is that it provides evidence that in type 1 diabetes, hyperglycemia may not be responsible for neuropathic changes on its own. The decrease in insulin and C peptide that accompanies hyperglycemia in type 1 diabetes may result in a loss of neurotrophic support that exacerbates the harmful effects of high glucose. Studies comparing animal models of type 1 and type 2 diabetes have demonstrated more severe sensory neuropathy (38) and autonomic neuropathy (10,14) in type 1 diabetes despite similar levels of hyperglycemia.

5. Differential responses of subpopulations of autonomic neurons to diabetes

5.1. Sympathetic neurons: in vivo studies

The question of differential vulnerability of subpopulations of autonomic neurons to diabetes has received most attention with respect to the development of neuropathic changes in CG/SMG but not SCG neurons (see reference 11 for review). In line with the observation of the glove-stocking distribution of peripheral neuropathy where the longest axons are affected to the greatest extent, one possible explanation considered was that the axons projecting from the CG/SMG are affected because they are longer than those projecting from the SCG. However, axons projecting from the sympathetic chain to supply mesenteric vessels are of equal length to those supplying the small intestine from the CG/SMG and they do not develop axonal dystrophy in diabetes. In addition, this could not explain the degenerative changes observed in intrinsic VIP-containing myenteric neurons where the axons only project short distances within the gastrointestinal tract. Differential influence of the target has also been considered since diabetes causes hypertrophy of the smooth musculature of the small intestine that is supplied by the CG/SMG. Together with deficient retrograde transport of NGF from the target, it was initially thought that loss of neurotrophic support for CG/SMG neurons accounted for the selective development of neuroaxonal dystrophy. However, a later study revealed that rather than reduced levels, NGF content was actually increased in the CG/SMG from diabetic rats whereas it was unchanged in the SCG (11).

This has led to the proposal that the intrinsic properties of sympathetic neurons may determine their susceptibility to diabetes. A comparison of gene expression in CG/SMG and SCG from rats has demonstrated that there are marked differences in gene expression between the two ganglia under normal conditions. Furthermore, diabetes induced considerably more changes in gene expression in CG/SMG than in SCG. Such changes in expression included genes involved in the structure and function of synapses and mitochondria and in oxidative stress (45). The activities of the antioxidant enzymes glutathione peroxidase and superoxide dismutase have been shown to be considerably lower in CG/SMG than in SCG under normal conditions (46). In addition, there is ultrastructural evidence that the blood–ganglion barrier is less effective in the CG/SMG (47). Thus, sympathetic neurons from different ganglionic sources cannot be regarded as a homogeneous population that would necessarily respond in the same way to harmful stimuli.

5.2. Sympathetic and myenteric neurons: in vitro studies

Studies of isolated CG/SMG and SCG neurons *in vitro* provide an approach in which a comparison can be made of their responses to high glucose or oxidative stress in the absence of hypoxia or the target changes that occur concurrently in diabetes *in vivo*. Primary cultures of neurons from adult rat CG/SMG and SCG have been investigated to avoid the confounding factor of developmental differences when using embryonic neurons. Neurons from both ganglia survived to

a similar extent in the absence of NGF. However, CG/SMG neurons took longer to initiate neurite outgrowth than SCG neurons and exposure to high glucose inhibited this process (46). Preliminary findings also indicated that NGF can protect SCG but not CG/SMG neurons against the harmful effects of high glucose (unpublished observations). Exposure of ganglion explants to menadione causes oxidative stress, as indicated by the accumulation of the major product of lipid peroxidation, 4-hydroxynonenal. CG/SMG neurons were much more sensitive to oxidative stress than SCG neurons. Significantly, low concentrations of menadione caused an accumulation of TH in CG/SMG but not in SCG neurons (48). Thus, oxidative stress *in vitro* resulted in the same differential accumulation of TH that occurs in diabetes *in vivo*. This provides evidence that the capacity of subpopulations of sympathetic neurons to withstand oxidative stress may be a major factor in determining their susceptibility to the development of autonomic neuropathy in diabetes. It is interesting to note that microtubules have been described as very susceptible to disruption by 4-hydroxynonenal, resulting in impaired axonal transport (49). Therefore, this provides a mechanism whereby oxidative stress could result in axonopathy without necessarily causing apoptosis. Using cytochrome c oxidase activity as a marker of metabolic activity, preliminary evidence has been obtained demonstrating that CG/SMG neurons display a higher range of metabolic activity than SCG neurons under normal conditions and that there is a positive correlation between accumulation of TH and high cytochrome c oxidase activity in CG/SMG neurons from diabetic rats (unpublished observations). Thus, it can be speculated that the level of metabolic activity within a neuron may influence the degree of oxidative stress that is induced under diabetic conditions.

In vitro studies have also been performed on whole-mount preparations of the myenteric plexus from adult rat ileum. Exposure to high glucose levels induces an accumulation of VIP and galanin in myenteric neuronal cell bodies, a response that is also observed in diabetes *in vivo* (50). This indicates that hypertrophy of the muscle, which does not occur *in vitro*, is not involved in the process. Interestingly, high glucose levels did not induce the reduction in the proportion of myenteric neurons expressing nNOS that is observed in STZ-diabetic rats (51, unpublished observations). In contrast, it has been reported that exposure of myenteric neurons to AGEs *in vitro* does result in the loss of nNOS expression (52). A particular association between the presence of nNOS and the harmful effect of AGEs has been observed in pelvic ganglion neurons where nNOS-containing neurons undergo apoptosis in diabetes whereas TH-containing neurons do not (13). Using a neuronal cell line, AGEs only initiated apoptosis in those cells in which the expression of nNOS had been induced (53). This raises the possibility that diabetes-induced changes in autonomic neurons may not always occur via the same mechanism and that the particular neurotransmitter that an autonomic neuron expresses may also influence its susceptibility to the development of neuropathy in diabetes.

5.3. Differential responses to treatment

It is now evident that subpopulations of autonomic neurons not only have a differential susceptibility to the development of neuropathy in diabetes but that they also display a differential response to treatment. Experimental studies on the sensory nervous system have demonstrated that treatments with NGF (32), α-lipoic acid (an antioxidant) (54), aminoguanidine (an inhibitor of AGE formation) (55), and aldose reductase inhibitors (56) can all prevent and/or reverse sensory deficits induced by diabetes. However, none of these agents are effective for treating diabetes-induced changes in CG/SMG neurons or their axons in the gastrointestinal tract (15,36,57,58). To date, the only treatment that has been reported to reverse neuroaxonal dystrophy in the CG/SMG has been IGF1 (44). In addition, a study of α-lipoic acid revealed that whereas treatment with this antioxidant did prevent the loss of nNOS expression in erectile tissue and the loss of NA in the heart induced by diabetes, at the same dose, it failed to prevent the accumulation of VIP in myenteric neurons or the loss of NA in the ileum that occurred in STZ-diabetic rats (58). These findings have clinical implications in the search for effective treatment of autonomic neuropathy in diabetic patients.

6. Summary

The significance of autonomic neuropathy in diabetes has been underestimated. Clinical studies have been hampered by the difficulty in establishing non-invasive techniques to evaluate autonomic function in specific target organs. Experimental studies have revealed a complex pattern of change in diabetes in which different populations of autonomic neurons respond differently in diabetes with only some undergoing degeneration. Characteristic neuropathic changes include accumulation of neurotransmitters within the neuronal cell body, loss of neurotransmitter in the nerve terminals with swelling of the varicosities and dystrophy. There is preliminary evidence that the intrinsic properties of autonomic neurons determine their response in diabetes. Increased metabolic activity and decreased antioxidant defence may predispose subpopulations of autonomic neurons to the development of neuropathy. Clinical studies investigating

potential therapies for preventing or reversing diabetic neuropathy need to consider that damage to autonomic nerves, particularly those supplying the gastrointestinal tract, may be more difficult to treat.

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