REVIEW

New insights in diabetic autonomic neuropathy in children and adolescents

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Abstract

Diabetic autonomic neuropathy (DAN) represents a major complication of diabetes mellitus but there is considerable uncertainty about its incidence, prevalence, pathogenesis, diagnosis, and prognosis. There are conflicting opinions about the pathogenesis of DAN: the 'classical hypothesis' has been supplemented by some new insights. Clinical symptoms of autonomic neuropathy do not generally occur until long after the onset of diabetes. DAN seems to be detectable even in asymptomatic children and adolescents with diabetes and is associated with the most serious consequences, such as cardiovascular dysfunction. Because of its association with a variety of adverse outcomes, including cardiovascular deaths, cardiovascular autonomic neuropathy is the most clinically important and well-studied form of DAN. No form of therapy in DAN has been identified that provides unequivocal, safe, and effective stabilization or reversal of the condition, just a near normal control of blood glucose in the early years after the onset of diabetes that may delay the development of clinically significant nerve impairment. This article reviews recent developments in knowledge of epidemiology, pathogenesis, clinical symptoms, diagnosis, and therapy of DAN.

European Journal of Endocrinology 161 811–818

Introduction

Diabetic autonomic neuropathy (DAN) is among the least recognized and understood complications of diabetes despite its significant negative impact on survival and quality of life in people with diabetes (1, 2). DAN can involve the entire autonomic nervous system (ANS). ANS vasomotor, visceromotor, and sensory fibers innervate every organ, and DAN may be either clinically evident or subclinical with dysfunctions of one or more organ systems (e.g. cardiovascular, gastrointestinal, genitourinary, sudomotor, or ocular) (3). The clinical presentation is most often a heterogeneous pattern of symptoms in different organs, which can lead to misdiagnosis (4–6). Nonetheless, there are also correlations to other long-term diabetic complications, such as nephropathy and retinopathy (7). Clinical symptoms of autonomic neuropathy do not generally occur until long after the onset of diabetes. Subclinical autonomic dysfunction can, however, occur within a year of diagnosis in type 2 diabetes patients and within 2 years in patients with type 1 diabetes (5). There is controversy over whether subclinical signs of autonomic dysfunction can be found in children with diabetes (8–15). Because of its association with

adverse outcomes, including cardiovascular deaths, cardiovascular autonomic neuropathy (CAN) is the most clinically important and well-studied form of DAN.

This article reviews recent developments in knowledge of epidemiology, pathogenesis, clinical symptoms, diagnosis, and therapy of DAN.

Epidemiology

Comparison of epidemiological studies of DAN is difficult because of inconsistent definitions, a lack of different diagnostic methods and different study selection criteria. The variance among prevalence studies also reflects the type and number of tests performed, and the presence or absence of signs and symptoms of autonomic neuropathy (16).

Among these studies, Ziegler *et al.* recruited a total of 1117 diabetic patients (647 patients with type 1 diabetes mellitus (T1DM) and 524 patients with T2DM) in 22 diabetes centers in Austria, Germany, and Switzerland in 1992 (16). In this study, more than two out of six autonomic function tests showed abnormal findings in 25.3% of patients with T1DM and in 34.3% of patients with T2DM. In our experience,

47 of 110 diabetic children and adolescents considered showed one or more abnormal test for cardiovascular autonomic dysfunction (11). In a prospective study, low sensory nerve conduction and autonomic dysfunction were found in ~25% of 144 diabetic children (17). Karavanaki & Baum (8) reported reduced papillary adaptation in darkness in 13.8% of children with diabetes compared with 5.8% of controls; 50% of these children also had impaired heart rate variation. Suys *et al.* (18) found evidence of cardiac dysfunction in a significant proportion of children and adolescents with diabetes: diabetic children had significantly longer QTc intervals and a significantly larger QTc dispersion than controls. Vazeou *et al.* (19) failed to find a correlation between autonomic function and gastro-intestinal symptoms in children and teenagers with diabetes, nor an effect of mild-to-moderate hyperglycemia on gastrointestinal motility. On the other hand, Toporowska-Kowalska *et al.* (20) showed derangements of the gastric myoelectrical activity in 71% of children with early stage T1DM. Boysen *et al.* (21) found pathologic cardiorespiratory reflexes for at least one item in 75% of the diabetic and 60% in the healthy control group.

These data suggest that DAN is a complication often underestimated and, therefore, it requires more standardized screening tests.

Pathogenesis

There are conflicting opinions about the pathogenesis of diabetic neuropathy. Well described mechanisms include excessive flux of polyols, particularly sorbitol through the aldose reductase pathway associated with depletions in nerve and Schwann cell of myo-inositol and rises in protein kinase C subunits, microangiopathy and hypoxia involving the peripheral nerve trunk, ganglia or spinal cord, oxidative and nitrative stress from free radicals, deficiency of growth factors, or their uptake and abnormal glycosylation of structural neuron proteins (22–24). In a subpopulation of individuals with neuropathy, immune mechanisms may also be involved (25).

The ‘classical hypothesis’ has been supplemented by some new insights summarized in Table 1.

Table 1 Pathogenesis of diabetic neuropathy.

Classical hypothesis	New mechanisms
Accumulation of polyols	Direct insulin signaling
Microangiopathy	C-peptide
Oxidative stress	RAGE
Deficiency of growth factors	PARP
Hyperglycemia	

PARP, poly (ADP-ribose) polymerase; RAGE, receptor for advanced glycation endproducts.

These include roles for direct insulin signaling on neurons and axons, which transduce growth signals, actions of the cleaved C-peptide product of the insulin prohormone, abnormal signaling by advanced glycation endproducts (AGEs) on neuronal and glial receptors and activation of poly (ADP-ribose) polymerase (PARP) in microvessels and neurons. Nonetheless, manipulation of these pathways may offer new therapeutic approaches (24).

Several experimental studies have investigated direct insulin signaling of sensory neurons in diabetic or injury models (26–39): the administration of local low-dose subhypoglycemic near nerve insulin reversed ipsilateral slowing of motor conduction velocity in diabetic rats without lowering plasma glucose levels. The local injections of insulin have no impact on changes of neuropathy in other territories of the same rat (contralateral side given carrier or in the caudal nerve) (27). Furthermore, low subhypoglycemic doses of insulin administered by an intrathecal infusion pump reversed motor and sensory nerve conduction slowing, myelinated axon atrophy and loss of skin innervations (26–28). Identical doses given subcutaneously did not improve neuropathy. Insulin given intrathecally is taken up by sensory neurons, binds their surface receptors, and can generate a cascade of intracellular molecules that support survival and outgrowth (26–30). Insulin receptors have been identified on mitochondria, and insulin can reverse their inappropriate depolarization during diabetes (31).

Changes resembling diabetes (slowing of conduction velocity and axonal atrophy) were generated by sequestering intrathecal insulin, using chronic infusion of an intrathecal antiinsulin antibody (26), suggesting the possibility that insulin itself, independently of its glycemic actions, is a source of important tropic support for peripheral neurons. A novel prospect is the possibility to administer insulin intranasally, an approach that can access the cerebrospinal fluid and has been used to treat other neurological conditions (29). Insulin is related to insulin-like growth factors in that they can cross-occupy each other’s receptors and share downstream survival transduction pathways (32–35).

C-peptide is a cleaved portion of the insulin prohormone, postulated to have independent actions on peripheral neurons (36). Deficiency of C-peptide may explain some of the changes observed in type 1 diabetes, in which C-peptide levels are absent, which are not observed in concurrent type 2 models with normal or elevated C-peptide levels but that have similar glycemic control (35, 37). Interruption of AGE–receptor for AGEs (RAGE) signaling is a novel strategy for attenuating diabetic complications. AGE ligation of RAGE inappropriately activates cellular second messengers (e.g. nuclear factor kappa B) (38–40). RAGE protein and mRNA are massively up-regulated in diabetic peripheral nerve and ganglia, whereas mice lacking RAGE are

protected from neuropathy (41). It may be possible to interrupt AGE–RAGE signaling on neurons by administering a soluble version of RAGE that sequesters circulating AGEs before they ligate neurons (38).

PARP is a DNA repair molecule that is activated by peroxynitrite, a free radical generated by nitric oxide and superoxide. Although its repair functions may be helpful, its overactivation depletes cellular energy stores and leads to apoptosis. PARP has been thought to contribute to the microangiopathy and its expression is up-regulated in diabetic neurons (42, 43).

Clinical manifestations

Clinical manifestations of autonomic dysfunction and other microvascular complications frequently occur concurrently but in inconsistent patterns (44). The ubiquitous distribution of ANS renders virtually all organs susceptible to autonomic dysfunction. Overt signs and symptoms of autonomic disease are described in Table 2.

CAN is the most prominent aspect because of the life-threatening consequences of this complication and the availability of direct tests of cardiovascular autonomic function. Reduced heart rate variation is the earliest indicator of CAN (45), and it is detectable at the subclinical stage (7, 46) and can, for instance, be clearly verified during deep respiration. Previous studies in more heterogeneous groups of patients with type 1

diabetes have demonstrated a reduction in various indices of heart rate variability (HRV) (47), suggestive of a reduction in vagal activity.

Reduced baroreflex sensitivity (BRS) seems to be a sensitive marker of autonomic cardiovascular dysregulation in a population of type 1 diabetic patients (48) and such a reduction in BRS has furthermore been repeatedly reported by different studies in type 1 and type 2 diabetic patients (49–52). A recent study (53) demonstrated a marked reduction of the spontaneous BRS in patients with type 1 diabetes, even in the absence of any clinically detectable complications. The reduction in BRS co-segregated with signs of sympathetic predominance. Nevertheless, the diabetic participants were able to increase BRS in response to slow deep breathing, an intervention capable of inducing an increase in BRS in healthy control participants. The opposite intervention, active standing, induced a similar marked reduction in both groups. In contrast, in patients with a denervated heart, BRS did not increase after slow breathing, suggesting that the abnormalities seen in the patients with type 1 diabetes could, at least to some extent, be considered functional.

Increased daytime blood pressure and reduced nocturnal dipping can already be found in children with T1DM. For Krause *et al.* (54), impaired BRS can cause this abnormal blood pressure behavior in children and adolescents with type 1 diabetes, reflecting an early stage of DAN.

Although cardiovascular integrity as a whole is well preserved in adolescents with T1DM, reactivity of the cardiovascular ANS system seems to fall behind that of the healthy control subjects. Although physiological maturation during adolescence is connected with the pubertal stages more closely than with age, maturation according to the Tanner pubertal stages is seldom taken into account (10, 12), while age has been used as a marker of puberty in earlier power spectral analysis of HRV and blood pressure (9, 55–57). Pubertal staging permits closer and more accurate observation of clinical and research variables. The inverse association between puberty and deep breathing test was observed in the boys but not in the girls with diabetes after adjusting for body mass index. This may be partly due to different body composition in boys and girls during puberty, or this might be true functional deterioration of ANS, since the above-mentioned association was not seen in the control boys. However, pubertal maturation, glycemic control, and duration of diabetes may be interrelated, which makes the interpretation of the analysis difficult. Puberty appears to enhance microvascular complications (58, 59), perhaps due to hormonal changes and insulin resistance (60); some studies show that metabolic control correlates with decreased HRV and heart rate reactivity (61), while others report no correlation (62). The influence of metabolic control on cardiovascular function in adolescent patients is controversial (63).

Table 2 Clinical manifestations of diabetic autonomic neuropathy.

Cardiovascular
Resting tachycardia
Exercise intolerance
Orthostatic hypotension
Silent myocardial ischemia
GI
Esophageal dysmotility
Gastroparesis diabeticorum
Constipation
Diarrhea
Fecal incontinence
Genitourinary
Neurogenic bladder (diabetic cystopathy)
Erectile dysfunction
Retrograde ejaculation
Female sexual dysfunction (e.g. loss of vaginal lubrication)
Metabolic
Hypoglycemia unawareness
Hypoglycemia-associated autonomic failure
Sudomotor
Anhidrosis
Heat intolerance
Gustatory sweating
Dry skin
Pupillary
Pupillomotor function impairment (e.g. decreased diameter of dark-adapted pupil)
Argyll-Robertson pupil

Additional symptoms such as exercise intolerance, orthostatic hypotension, and an increasing limitation in HRV are the manifestations of progressive damage to the autonomic balance (7).

Diagnosis

Quantitative tests of autonomic function have historically lagged behind measures of motor nerve function and sensory nerve function deficits. The lack of interest in the development of such measures was partly due to the erroneous view that DAN was only a small and relatively obscure contributor to the peripheral neuropathies affecting individuals with diabetes (64).

Quantification of autonomic function is based on a series of tests, reported in Table 3, detecting the presence of CAN, gastrointestinal, genitourinary, and pupillary dysfunction.

In particular, regarding CAN, in the early 1970s, Ewing *et al.* (65, 66) proposed five simple noninvasive cardiovascular reflex tests:

- i. Falling systolic blood pressure in response to standing.
- ii. Heart rate response to standing (30/15 ratio).
- iii. Heart rate response during deep breathing (expiration:inspiration ratio).
- iv. Heart rate response to Valsalva maneuver.

Table 3 Diagnosis of diabetic autonomic neuropathy.

Assessing cardiovascular function
Heart rate variability (HRV)
Ewing battery
• Falling systolic blood pressure in response to standing
• Heart rate response to standing (30/15 ratio)
• Heart rate response during deep breathing (expiration:inspiration ratio)
Heart rate response to Valsalva maneuver
ECG (QTc)
Assessing GI autonomic function
Gastroduodenoscopy
Manometry
Double-isotope scintigraphy
Electrogastrography
Anorectal manometry
Assessment of colonic segmental transit time
Assessing genitourinary autonomic function
Urinary culture
Postvoid ultrasound
Cystometry
Sudomotor assessing sudomotor function
Quantitative sudomotor axon reflex test
Sweat imprint
Thermoregulatory sweat test
Sympathetic skin response
Assessing papillary
Reflex response to light

One abnormal test of this battery is regarded as a sign of early autonomic dysfunction (67, 68).

Another test is downward tilting BRS test (79): electrocardiogram (ECG) and continuous beat-to-beat blood pressure measurements are registered in the supine position for 15 min. The mean regression coefficient of falling and rising sequence is the BRS; BRS <10 mmHg/ms is considered pathologic (70–72).

Earlier consensus statements indicate that testing for the prolongation of Bazett’s heart rate-corrected QT interval (QTc) is easy and specific for diabetic autonomic failure (73). The QT interval reflects the total duration of ventricular myocardial depolarization and repolarization. The relation between a prolonged QT interval and an increased risk of sudden death has been extensively explored in familial long QT syndrome, sudden infant death, and ischemic heart disease, as well as in adults with diabetes mellitus (74–77). Several studies describe the relation between QTc interval prolongation, diabetic complications, and an increased mortality rate in adults (78, 79). Therefore, Suys *et al.* (80) demonstrated a QTc prolongation and a larger QTc dispersion are already present in a significant proportion of children and adolescents with diabetes.

Therapeutic approaches

Prevention

One of the most important components of etiology-based treatment is the stabilization of glycemic control. The beneficial effect of intensive treatment, proven by the diabetes control and complications trial (DCCT) and the UK Prospective Diabetes Study, has been confirmed by new data in the recent years. During the follow-up of the DCCT cohort as part of the Epidemiology of Diabetes Intervention and Complications study, every type 1 diabetic patient was encouraged to apply intensive insulin treatment (81). It was clearly shown that the beneficial effect of 6.5 years of intensive therapy on the development and progression of the neuropathy persisted for at least 8 years after completion of the DCCT as the prevalence of neuropathy then still differed in the different groups despite the similar intensive treatment during the follow-up period. It should be emphasized that a slower progression, not an improvement of the neuropathy, was achieved (82). In the EURODIAB IDDM Complications Study and the EURODIAB Prospective Study, the development and progression of neuropathy were found to be strongly related to increased cardiovascular risk factors including the cholesterol/high-density lipoprotein cholesterol ratio and the triglyceride levels (83, 84). These observations underline the importance of strict glycemic control and multifactorial intervention.

Secondary interventions

No form of therapy in DAN has been identified which provides unequivocal, safe, and effective stabilization or reversal of the condition. There have been many trials, most providing only modest disease stability or no improvement.

The polyol hypothesis has generated trials of aldose reductase inhibitors (ARIs) over the past several decades. Although several compounds were investigated in clinical trials in the 1980s and 1990s, the results were rather disappointing (82). Studies with sorbinil, tolrestat, and zenarestat showed serious adverse events (82), while there was a lack of efficacy with ponalrestat and zopolrestat (85). However, ARI trials are still very much in the research area and, although there are some promising reports on improvement in symptoms and in some objective measures of neuropathy, the degree of benefit obtained has not been outstanding, and therefore, it is still too early to evaluate the role of ARIs in the management of diabetic neuropathy, especially in children and adolescents. A recent Cochrane review of ARIs, nevertheless, concluded that benefit has not been demonstrated from these agents (86).

The impact of α -lipoic acid, a potent antioxidant, has been analyzed in several trials. A meta-analysis of i.v. therapy concluded that there were improvements in positive neuropathic symptoms and neuropathic deficit (87). Recently, oral controlled-release formulation of α -lipoic acid was developed, but it was not an effective treatment for decreasing oxidative damage in T1DM (88).

C-peptide is a cleaved portion of the insulin prohormone, postulated to have independent actions on peripheral neurons. Although its exact mechanism of action is uncertain, it may sensitize insulin receptors. Deficiency of C-peptide may explain some of the changes observed in models of type 1 diabetes, in which C-peptide levels are absent, which are not observed in concurrent type 2 models with normal or elevated C-peptide levels but that have similar glycemic control (35, 89). Early clinical trials in patients with type 1 diabetes have demonstrated benefits in sensory conduction, neurological impairment scores, and vibration perception (90).

The experimental data indicate that the lack of several physiologic actions of C-peptide mediated via nitric oxide-related mechanisms may lead to neuropathy (91). Following a 3-month period of subcutaneous treatment with C-peptide, the respiratory HRV was increased in patients with type 1 diabetes in comparison with the placebo (92). Another randomized study confirmed the efficacy of C-peptide on sensory nerve conduction velocity in patients with type 1 diabetes without symptoms of neuropathy and with <10-year duration of the disease (36).

The expectations from gene therapy are high. The use of the herpes simplex virus vector-mediated gene

transfer of the vascular endothelial growth factor in a mouse model of diabetic neuropathy provided promising results on the prevention of neuronal dysfunctions (93).

Another reliable option for achieving long-term insulin independence is whole pancreas transplantation. The proposed benefits of pancreas transplantation are clear: improved quality of life, prevention of recurrent diabetic nephropathy, freedom from exogenous insulin with euglycemia and normalization of glycosylated hemoglobin, less stringent dietary restrictions, less frequent blood glucose monitoring, and stabilization of or improvement in secondary complications. The tradeoffs to the patient are the operative risk, the need for chronic immunosuppression, and the inherent side effects of chronic immunosuppression (94).

Stem cells might represent a potential source of tissues for cell therapy protocols, and diabetes is a candidate disease that may benefit from cell replacement protocols. The pathology of type 1 diabetes is caused by the autoimmune destruction or malfunction of pancreatic β -cells, and consequently, a lack of insulin. The absence of insulin is life-threatening, requiring diabetic patients to take daily hormone injections from exogenous sources. However, insulin injections do not adequately mimic β -cell function; this results in the development of diabetic complications. New possibilities have been opened by embryonic and adult stem cells in regenerative medicine for the cure of diabetes (95).

Conclusions

DAN is a common complication of diabetes that is often associated with considerable morbidity and mortality. The epidemiology and natural history of DAN are still uncertain, largely because of confusion regarding the definition and measurement of this disorder. The best evidence suggests that near normal control of blood glucose in the early years after onset of diabetes may delay the development of clinically significant nerve impairment, and therefore, children and adolescents with diabetes are critical targets for primary prevention of this complication.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

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Received 14 August 2009

Accepted 23 August 2009