Cardiac autonomic activity and Type II diabetes mellitus

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ABSTRACT
CAN (cardiac autonomic neuropathy) is a common complication of diabetes. Meta-analyses of published data demonstrate that reduced cardiovascular autonomic function, as measured by heart rate variability, is strongly associated with an increased risk of silent myocardial ischaemia and mortality. A major problem in ischaemia-induced impairment of vascular performance in the diabetic heart is unrecognized cardiac sympathetic dysfunction. Determining the presence of CAN is based on a battery of autonomic function tests and techniques such as SPECT (single-photon emission computed tomography) and PET (positron emission tomography). Nevertheless, spectral analysis of heart rate variability seems to remain the primary technique in evaluating CAN, due to its low cost, easy use and good intra-individual reproducibility.

CARDIOVASCULAR AUTONOMIC ACTIVITY
History
Sympathetic and parasympathetic innervations in the heart play a major role in the regulation of cardiac function. The existence of sensory nerve endings in the heart was first suggested in 1894 [1], although Wollard [2], in 1926, concluded that a large portion of cardiac sensory endings were of vagal origin. Indeed, Wollard [2] observed that an experimental bilateral stellectomy did not markedly modify what he considered to be the normal aspect of the sensory supply to the heart. Subsequently, Holmes [3] observed survival of the ‘terminal nervous network’ after vagotomy, thus hypothesizing that sympathetic afferent fibres were likely to be implicated. In 1963, Khabarova [4] observed that “afferent fibers of spinal type innervate the same regions and layers of the heart as the vagal fibers, and their afferent fibers and endings frequently lie side by side with afferent fibers and endings of the vagus nerve”. This opinion has the merit of agreeing with the electrophysiological findings which, so far, have suggested that vagal and sympathetic sensory nerve endings are intermingled in all regions of the heart.

Anatomy
Great progress has been made over the past two decades in identifying the central pathways and neurotransmitters that regulate the cardiovascular system, particularly those that subserve the short-term reflex control of sympathetic activity. The importance of the hypothalamus and other forebrain regions in cardiac regulation has been recognized for many years, but relatively little is known about the functional organization of forebrain mechanisms that regulate the cardiovascular system, both in the short- and long-term. Much more attention is now being paid to define these forebrain mechanisms. In particular, it is now clear that these central mechanisms can be up- or down-regulated in response to long-term physiological or parapathophysiological stimuli, such as exercise training [5], changes in environmental temperature [6], heart failure [7], hypertension [3] and diabetes [8] (Figure 1).

Key words: autonomic nervous system, cardiac autonomic neuropathy, heart rate variability, Type II diabetes mellitus.
Abbreviations: CAN, cardiovascular autonomic neuropathy; CVD, cardiovascular disease; DAN, diabetic autonomic neuropathy; HF, high frequency; HRV, heart rate variability; LF, low frequency; MIBG, [123]I-meta-iodobenzylguanidine; NE, noradrenaline; NEFA, non-esterified fatty acid; NO, nitric oxide; PET, positron emission tomography.
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HOW CAN WE STUDY AUTONOMIC CARDIAC ACTIVITY?

Quantitative tests of autonomic function have historically lagged behind measures of motor and sensory nerve function deficits. The lack of interest in the development of such measures was partly due to the erroneous view that autonomic neuropathy was only a small contributor to neuropathy. In the early 1970s, Ewing et al. proposed five simple non-invasive cardiovascular reflex tests that have been applied successfully in many studies (work reported in 1985 [9]). In fact, many studies using these tests have provided information on the prevalence of DAN (diabetic autonomic neuropathy), its natural history, clinical prognosis and relationship with other chronic diabetic complications. Among the clinical reflex tests, the tests used most widely, validated and best known in their physiological bases are heart rate variation on deep breathing and lying-to-standing, the Valsalva manoeuvre and BP (blood pressure) response to standing. In addition to traditional cardiovascular reflex tests, other methods have been developed, such as plasma NE (noradrenaline) and NE spillover, spectral analysis of HRV (heart rate variability), which assesses indirectly cardiac autonomic dysfunction, PET (positron emission tomography), which is the first technique able to provide a direct characterization of the pattern and extent of cardiac sympathetic dysfunction [10], and MIBG ([123]I-meta-iodobenzylguanidine) scintigraphy, which is a recent non-invasive method for the in vivo evaluation of sympathetic activity through measurement of postganglionic presynaptic noradrenergic uptake [11]. With regard to plasma NE and NE spillover, their indiscriminate use as markers of sympathetic nervous activation may be misleading in specific physiological events. However, despite the ability of PET and MIBG to directly characterize cardiac autonomic dysfunction, spectral analysis appears to remain the primary technique in evaluating cardiac autonomic dysfunction due to its low cost, ease of use and good intra-individual reproducibility. The main advantage of spectral analysis is the possibility of assessing not only the amount of overall variability, but also the frequency-specific oscillations and the relative impact on variability of sympathetic and vagal modulation in the heart.

Two major oscillatory components are usually detectable in spectral analysis of which one, synchronous with respiration, is described as HF (high frequency; 0.18–0.40 Hz and varying with respiration) and is generally considered a marker of vagal activity, whereas the other, corresponding to the slow waves of arterial pressure, is described as LF (low frequency; 0.03–0.15 Hz) (Figure 2) [11,12]. The latter component seems to depend on more complex mechanisms. Maneuuvres enhancing the sympathetic drive or pathological condition associated with sympathetic hyperactivity lead to a marked relative increase in the LF component [13]. Some disagreement in its relationship with sympathetic tone is due to the observation that both LF and HF are reduced after atropine. Nevertheless, the hypothesis that LF can be influenced by the vagus is valid only if LF is evaluated in absolute and not in relative terms. Thus it is more appropriate to consider the relationship between LF and HF (LF/HF ratio) in terms of sympatho-vagal balance, rather than consider them separately as independent indices of...
sympathetic and vagal activity. In fact, several studies [8–13] have confirmed that the LF/HF ratio offers an acceptable indication of the autonomic vagal-sympathetic balance in heart control. Previous studies have demonstrated that an unbalanced sympathetic/parasympathetic tone, with a prevalence of sympathetic activity, is associated with higher cardiovascular mortality in Type II diabetic patients [14–17]. Such unbalanced sympathetic/parasympathetic tone can be responsible for many cases of sudden death [14–17] in diabetic patients, despite the absence of documented pre-existing heart disease [16].

**CAN (CARDIOVASCULAR AUTONOMIC NEUROPATHY) AND DIABETES**

DAN is among the least recognized and understood complication of diabetes, despite its significant negative impact on survival and quality of life in people with diabetes [18,19]. The metabolic disorders of diabetes lead to diffuse and widespread damage of peripheral nerves and small vessels. One of the most overlooked complications of diabetes is CAN [20]. CAN results from damage to the autonomic nerve fibres that innervate the heart and blood vessels and it causes abnormalities in heart rate control and vascular dynamics [21]. Reduced heart rate variation is the earliest indicator of CAN [22]. The link between diabetes mellitus and CVD (cardiovascular disease) is well established and recognized. Recently, in the National Cholesterol Education Program (NCEP), diabetes has been considered a factor for cardiovascular risk development [23]. Thus it is recommended that greater precautionary measures, similar to those for established CVD, should be taken in patients with diabetes. These changes are based on findings showing that CVD occurs at a significantly higher rate in individuals with diabetes than in the general population [24–26]. In particular, the Nurse's Healthy Study has provided evidence of increased risk of CVD events before diagnosis of diabetes, with risk levels increasing further after diabetes diagnosis [24]. The authors also found that cardiovascular risk levels remained elevated even after adjustment for obesity and family history of diabetes. Compared with non-diabetic individuals, patients with diabetes carry a greatly increased risk not only of sustaining cardiovascular events, but also of poor outcomes associated with CVD. Several studies have provided evidence for an increased mortality risk among diabetic individuals with CAN compared with individuals without CAN [27,29]. Ewing et al. [27] reported a 2.5 year mortality rate of 27.5% that increased to 53% after 5 years in diabetic patients with an abnormal autonomic function test compared with a mortality rate of only 15% over the 5 year period among diabetic patients with normal autonomic function test results. A study by O'Brien et al. [28] reported 5 year mortality rates of 27% in patients having asymptomatic autonomic neuropathy compared with an 8% mortality rate in diabetic subjects with normal autonomic function tests. Rathmann et al. [29] reported the results of a study designed to assess the risk of mortality due to CAN among patients with CAN but without a clinical manifestation of severe complications (proteinuria, proliferative retinopathy, coronary heart disease or stroke) 8 years after their first clinical examination. In this study [29], the mortality of diabetic patients with CAN increased steadily over the 8 year period compared with an age-, sex- and duration of diabetes-matched control group where there was a death. Autonomic dysfunction was found to be an independent risk factor with a poor prognosis. Despite the increased association with mortality, the causative relationship between CAN and the increased risk of mortality has not been established conclusively. Several mechanisms have been suggested as being responsible for autonomic dysfunction and, among these, hyperinsulinaemia/insulin resistance seems to play a crucial role. There is strong evidence that acute physiological and pharmacological (euglycaemic clamp) increments in plasma insulin concentration stimulate sympathetic activity, as determined by measurements of venous plasma catecholamines concentration [30], plasma NE spillover [31] or direct micro-neurographic recordings of sympathetic nerve action potentials targeted at the skeletal muscle vasculature [32]. In particular, short-term infusion of insulin and chronic
hyperinsulinaemia induce a re-setting in cardiac autonomic control, mainly secondary to an increase in sympathetic activity [33–35]. These findings strengthen the hypothesis that hyperinsulinaemia/insulin resistance is implicated directly in the pathogenesis of cardiovascular mortality associated with Type II diabetes mellitus [35] through sustained overactivation of the cardiac sympathetic nervous system. Over the past decade, evidence has been accumulated indicating a double mechanism of action of insulin: a central neural action and a peripheral action. Insulin crosses the blood–brain barrier [36–38], and insulin receptors have been found in several distinct regions of the central nervous system such as the median hypothalamus [37]. The peripheral effects of insulin, at the cardiac sympathetic level, are mediated by NEFAs (non-esterified fatty acids) and the NO (nitric oxide)/\(l\)-arginine pathway. Insulin-resistant states are characterized by alteration in both of these functions. Recent evidence indicates that these two regulatory systems interact closely and that a defect in NO synthesis and an increase in plasma NEFAs may have an important role with regard to sympathetic action (Figure 3).

NO release accounts for the vasodilator action of insulin [39]. In vitro, insulin activates \(l\)-arginine transport and stimulates NO release in cultured vascular endothelial cells [40]. In vivo, insulin-induced vasodilation is abolished by a stereospecific inhibitor of NO synthase, \(l\)-NMMA (\(N^G\)-monomethyl-\(l\)-arginine), and by an inhibition of the synthesis of tetrahydrobiopterin, a cofactor necessary for NO synthesis [41,42]. Insulin may stimulate NO release either by a direct local effect on the vascular endothelium or by stimulating sympathetic nitricergic fibres. Comparison of vasodilation during local intrarterial and systemic intravenous insulin infusion has provided conflicting results [39]. Insulin stimulates NO release and blood flow in the denervated limb in patients who have undergone regional sympathectomy, indicating that it stimulates blood flow by a direct action at the vasculature [43]. Consistent with this hypothesis, insulin causes hypotension in patients with autonomic failure [44]. In innervate limbs, however, stimulation of sympathetic vasodilator outflow by insulin appears to be necessary to induce vasodilation, because the prevention of insulin-induced sympathetic activation by dexamethasone abolished the insulin-induced vasodilation [45]. The sympathetic nervous system modulates insulin-induced vasodilation, as indicated by the much more rapid vasodilation in the denervated compared with the innervated limb in patients with regional sympathectomy [46]. Moreover, it is possible that there exists a balance between the central neural sympatho-excitatory (via stimulation of neural peptide release) and sympatho-inhibitory (by stimulating NO release) action of insulin, as NO inhibits central neural sympathetic vasoconstrictor outflow [47,48]. Thus it is possible to conclude that insulin causes vasodilation by stimulating release of NO through a direct local effect on the vasculature and by stimulating neural vasodilator outflow. The sympathetic vasoconstrictor tone restricts insulin-induced vasodilation, and the mechanisms causing this sympathetic overactivity are not known. Hyperinsulinaemia is a candidate mechanism, but it is not invariably

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**Figure 3** Effect of hyperinsulinaemia/insulin resistance on the cardiac autonomic nervous system
Figure 4 Comparative effects of insulin, NEFAs (FFA) and vitamin E on LF, HF and LF/HF in patients with Type II diabetes

*P < 0.05, **P < 0.005 and [#P < 0.001 compared with baseline.

associated with sympathetic overactivity in man, as demonstrated by the normal sympathetic nerve activity in patients with insulinoma [49,50], and alternative mechanisms need be considered. NO inhibits central neural vasoconstrictor outflow in animals [51,52] and humans [47,48]. It is therefore possible that the defect in NO synthesis found in many insulin-resistance states [53] may contribute to sympathetic overactivity. This defect in NO synthesis could be acquired and/or inherited. With regard to an inherited defect, recent studies indicate that polymorphisms in eNOS (endothelial NO synthase) are risk factors for CVD associated with insulin resistance [54]. Thus it is possible to hypothesize that a defect in NO synthesis could contribute to both impaired insulin-induced vasodilatation and sympathetic overactivity characteristic of an insulin-resistance state.

More recently, the role of plasma NEFAs has also been stressed. In fact, elevated plasma NEFA levels might disrupt cardiac plasma membrane structure and function and raise intracellular calcium concentrations [55,56], thus affecting cardiac activity. We were able to demonstrate that cardiac sympathetic overactivity occurs in Type II diabetic patients by raising plasma NEFA concentrations [57]. In contrast, the same group of patients submitted to intensive insulin treatment to improve metabolic control had a secondary decline in plasma NEFA levels and a decrease in cardiac sympathetic nervous system activity [57]. Moreover, more recently, we have demonstrated that increased post-prandial plasma NEFA concentrations are associated with an enhanced degree of oxidative stress and an increased LF/HF ratio, an index of cardiac sympathovagal balance [58]. Such data seem to be particularly relevant in explaining the relationship between plasma NEFAs, oxidative stress and sudden death in Type II diabetic patients. In fact, it has been shown that increased plasma NEFA concentrations are a pro-oxidant factor [58] (Figure 4).

IS THERE AN APPROPRIATE THERAPY FOR CAN?

Timely identification of autonomic dysfunction in diabetic patients may expedite end-organ prophylaxis. In addition, improved nutrition and reduced alcohol and tobacco consumption are options available to patients with diabetes who have been identified with cardiac autonomic dysfunction. Substantial amelioration of metabolic control has been shown to be effective in both primary and secondary prevention. Nevertheless, optimization of glucose control is only a part of a successful multifactorial approach for treatment of neuronal and vascular impairment of cardiac performance in diabetes mellitus. Special interest has been aroused by results obtained using antioxidants. Early identification of CAN permits timely intervention with the antioxidant α-lipoic acid, which appears to slow or reverse progression of neuropathy in some studies [59]. Other antioxidants, such as vitamin E, have been shown to improve oxidative stress in patients with Type II diabetes, and this effect seems to be associated with a decrease in plasma catecholamine concentration and sympathetic nervous system activity [60]. In fact, chronic vitamin E administration improves the cardiac sympathetic balance in Type II diabetic patients [60]. Finally, the recent use of metformin appears to be promising. In fact, we have demonstrated [61] that the metformin-related decrease in plasma NEFAs and insulin resistance is associated with an improvement in cardiac autonomic nervous balance in overweight Type II diabetic patients. Nevertheless, only further studies on large numbers of patients can confirm the protective effects of metformin on cardiovascular autonomic dysfunction.

CONCLUSIONS

CAN is still a clinical entity which needs to be investigated. HRV has shed light on this pathophysiological problem, but several questions still need to be addressed. In light of this, the therapeutic approach remains to be defined, even if an improved metabolic control appears to play a key role. Further studies are needed to identify a clear marker of CAN as well as the therapeutic efficacy of any drug.

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