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Review

Diabetic cardiac autonomic neuropathy: Insights from animal models

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ABSTRACT

Cardiac autonomic neuropathy (CAN) is a relatively common and often devastating complication of diabetes. The major clinical signs are tachycardia, exercise intolerance, and orthostatic hypotension, but the most severe aspects of this complication are high rates of cardiac events and mortality. One of the earliest manifestations of CAN is reduced heart rate variability, and detection of this, along with abnormal results in postural blood pressure testing and/or the Valsalva maneuver, are central to diagnosis of the disease. The treatment options for CAN, beyond glycemic control, are extremely limited and lack evidence of efficacy. The underlying molecular mechanisms are also poorly understood. Thus, CAN is associated with a poor prognosis and there is a compelling need for research to understand, prevent, and reverse CAN.

In this review of the literature we examine the use and usefulness of animal models of CAN in diabetes. Compared to other diabetic complications, the number of animal studies of CAN is very low. The published studies range across a variety of species, methods of inducing diabetes, and timescales examined, leading to high variability in study outcomes. The lack of well-characterized animal models makes it difficult to judge the relevance of these models to the human disease. One major advantage of animal studies is the ability to probe underlying molecular mechanisms, and the limited numbers of mechanistic studies conducted to date are outlined. Thus, while animal models of CAN in diabetes are crucial to better understanding and development of therapies, they are currently under-used.

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Clinical Aspects of CAN in Diabetes

Autonomic innervation is one of the primary control mechanisms regulating heart rate and overall cardiac output (Pop-Busui, 2010). In CAN, these autonomic nerve fibers in the heart are damaged (Maser et al., 2003), resulting in a disruption of cardiac control (Spallone et al., 2011). CAN is detected in approximately 20% of diabetic individuals, increasing to 35%–65% in older patients with prolonged duration of diabetes (Spallone et al., 2011).

Clinically, CAN manifests itself in resting tachycardia, exercise intolerance, orthostatic hypotension, and reduced heart rate variability (HRV) (Vinik et al., 2003). A major meta-analysis showed a significantly increased mortality rate in diabetic patients with CAN compared to those without (Maser et al., 2003). There is also evidence for a link between CAN and cardiovascular mortality (Astrup et al., 2006; Spallone et al., 2011). Abnormalities in HRV and QT indices (measured by electrocardiogram (ECG)) serve as strong predictors of mortality independent of any other risk factors a diabetic patient may have (Lykke et al., 2008; Ziegler et al., 2008). However, some patients with CAN appear asymptomatic, with many of them remaining asymptomatic until CAN is in its most severe stages (Abd El Dayem et al., 2011; Low et al., 2004). CAN is also thought to contribute to high

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mortality risk through its relationship to silent myocardial ischemia (SMI). SMI is present in 20% of those with CAN and only present in 10% of those diabetic patients without CAN (Spallone et al., 2011). Patients with CAN have a high risk of major cardiac events, and those with both CAN and SMI have an even higher risk (Valensi et al., 2001).

Glycemic control, duration of diabetes, age, and hypertension are key factors that contribute to the development of CAN (Stella et al., 2000; Witte et al., 2005). Although some patients show no signs of CAN, there are indicators that put individuals at higher risk for CAN onset and progression. In the case of type I diabetes, poor glycemic control increases risk (Spallone et al., 2011). The same is true for type II diabetes, but risk is also affected by conditions such as dyslipidemia and obesity (Spallone et al., 2011). Studies have also indicated that smoking, high levels of HDL cholesterol, cardiovascular disease, waist circumference, and use of high blood pressure medications can affect one's risk for developing CAN (Spallone et al., 2011).

Diagnosing CAN is not yet a definitive process, but in general CAN progression can be characterized through various tests. These include HRV tests, postural blood pressure testing, and Valsalva maneuvers, which require the patients to exhale while maintaining pressure (Valensi et al., 2001), testing a complex set of reflexes involving both sympathetic and parasympathetic pathways to the heart and baroreceptors in the chest and lungs (Abd El Dayem et al., 2011; Vinik et al., 2003). It has been determined that one abnormal cardio-vagal test is enough to identify the possible presence of CAN or early CAN, but two abnormal cardio-vagal tests are required to confirm CAN in a patient (Spallone et al., 2011). Once CAN is confirmed, its progression into severe and advanced stages is determined by the presence and severity of orthostatic hypotension as well as more abnormal heart rate test results (Spallone et al., 2011). A reduction in HRV is one of the earliest signs of CAN, and is recommended to be evaluated in all patients with diabetes (Vinik et al., 2003). Beginning HRV monitoring immediately upon diagnosis of diabetes provides a baseline so that repeat tests can be taken at one year intervals (Vinik et al., 2003), serving as the earliest indicator and predictor of CAN onset (Abd El Dayem et al., 2011).

Once CAN is identified, there is no immediate treatment other than to monitor progression and to manage complications of CAN. However, several approaches to combat the effects of CAN are currently being studied. Certain angiotensin converting enzyme inhibitors (ACE inhibitors) have been shown to have beneficial effects on preventing cardiac autonomic complications of diabetes (Kontopoulos et al., 1997), but there are contradictory results (Malik et al., 1998) and more follow-up studies are needed (Vinik et al., 2003). Similar results for improving the effects of CAN have been found with beta-blockers (Ebbehoj et al., 2002; Vinik et al., 2003). Moreover, alpha-lipoic acid (ALA) taken orally was shown to improve CAN slightly by improving HRV (Ziegler et al., 1997). Further studies were performed to show the same type of effects of ALA from the use of vitamin E and C-peptide (Manzella et al., 2001; Spallone et al., 2011). Nevertheless, many more studies need to be done in order to confirm positive effects of these therapeutic agents. Despite the need to discover effective drugs and therapeutic methods to improve the prognosis of CAN and its complications, most of the current success comes from tight metabolic and glycemic control, as well as being aware of and managing potential symptoms and risk factors (Spallone et al., 2011; Vinik et al., 2003).

Studies Using Animal Models of CAN in Diabetes

Animal studies related to diabetic autonomic neuropathy in the heart to date largely fall into one of three categories: 1) visualization of the anatomy of the cardiac autonomic nerves by immunohistochemistry (IHC) or electron microscopy (EM); 2) functional properties of the nerves (norepinephrine (NE) concentration in the tissue, NE release, or uptake of a radiolabeled NE mimetic); or 3) functional effects on the heart (changes in heart rate and HRV). Cardiac contractility, and subsequently, cardiac output, are also likely to be affected by changes in the function of autonomic nerves during diabetes. However, it is not easy to differentiate between diabetes-induced changes in cardiac autonomic nerves and the well-documented effects of diabetic cardiomyopathy, in which the major effect is directly on the cardiomyocytes themselves. Diabetic cardiomyopathy has been extensively reviewed elsewhere (Boudina and Abel, 2010; Poornima et al., 2006), and studies examining diabetes-induced changes in contractility will not be discussed here unless specifically targeted at examining the role of the neuropathy.

Characterization of CAN in Animal Models

One of the aims of characterizing CAN in animal models is to better understand the disease pathology. In order to do this it is crucial to be able to determine in what ways the animal models are similar to the human disease, and in what ways they are different. However, as explained above, the diagnosis of CAN in humans is not by a single definitive test but by a combination of several suggestive tests. Many of these human tests require communication with and cooperation from the patient, for example remaining still during HRV testing, undergoing tilt-table testing for orthostatic hypotension, or using specialized equipment/following specific instructions during the Valsalva maneuver. These are clearly not possible in animal studies, making it difficult to assess the relevance of any characterization in animals to human disease. However, there are many characterization studies that can be done in animals that are impossible in humans because they require terminal procedures. For example, characterizing the anatomy of the damaged cardiac nerves by IHC or EM requires removal and processing of the heart. While this may be possible in post-mortem studies in humans, animal studies allow for detailed characterization under strictly controlled conditions. Another major advantage to animal studies is that the characteristics of CAN may be determined over the timescale of disease progression.

Regardless of whether or not characterization of CAN in animals will reveal anything new about the human disease, a second, extremely important reason to characterize diabetic CAN in these animals is to determine if and how they may be useful as models of the disease state, since having a variety of even partially-relevant animal models of CAN allows us to probe the mechanisms underlying the disease through intervention studies (for example, using pharmacological tools or genetic manipulation). These studies are where the real advantages of animal models become apparent, but they are only useful if the models have been well-characterized and shown to be relevant to human disease.

Compared to other diabetic complications, such as nephropathy, retinopathy, and even peripheral sensory neuropathy, the numbers of animal studies examining diabetic CAN are very low. Those studies that do exist span a wide range of species, methods of induction of diabetes, timescales, and methods of assessment. A summary of the relevant publications is shown in Table 1.

A handful of studies have quantified innervation of hearts using IHC. A consistent finding has been a reduction in ventricular sympathetic staining: in db/db mice at 6 months of age (Tessari et al., 1988); in STZ-treated mice after 6 months of diabetes (Kellogg et al., 2009); in BioBreeding/Ottawa Karlsburg (BB/OK) rats after 6 months of diabetes (Schneider et al., 2010); and in alloxan-treated rabbits 20 weeks post-treatment (Wang et al., 2012). In parallel to the IHC, Wang et al. also showed a reduction in tyrosine hydroxylase (TH) expression by quantitative PCR. Mabe and Hoover (2011) looked at the atrial innervation using markers of both sympathetic (TH) and parasympathetic (choline acetyltransferase, ChAT, and vesicular acetylcholine transporter, VAChT) nerves. They found an increase in VAChT-positive neurons in the sinoatrial (SA) node of STZ-treated mice after 8 and 16 weeks of diabetes, but no changes in innervation of the atria. In contrast to this finding, Yang and Chon (2011) showed reduced synaptophysin-positive neurons (a marker of general nerves) in the SA node of Akita mice at 4 months of age. However, the differences in strain of mouse, cause of diabetes, and type of staining, not to mention the low number of studies,