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Diabetes and Cardiovascular Autonomic Dysfunction: Application of Animal Models

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Abstract

When diabetes is associated with cardiovascular autonomic dysfunction, there is a poor prognosis and increased morbidity and mortality. Information on the mechanisms of diabetes-associated autonomic dysfunction has been provided by advanced studies using physiological, pharmacological, anatomical and molecular methods in experimental animal models of insulin deficiency and resistance. This has been augmented by new approaches which combine diabetes induction with genetically modified animal models. The aim of this review is to outline and discuss the animal models used for the study of insulin deficiency and insulin resistance with a focus on autonomic neural interactions. The goal is to better understand the clinical relevance of cardiovascular autonomic dysfunction associated with diabetes.

Keywords

autonomic neuropathy; diabetic animal models; blood pressure; heart rate; diet; obesity

1. INTRODUCTION

Diabetes mellitus has reached epidemic levels in the United States (US) and worldwide. The prevalence estimate by the Center for Disease Control (CDC) in 2008 is 8% of the US population or 24 million people. It is of particular concern that the disease incidence is expected to increase worldwide by more than 100% between 2000 and 2030 (Wild *et al.*, 2004). Clinical issues related to the disease are focused not only on glucose metabolism, but also on cardiovascular pathologies which are the likely cause of morbidity and mortality. It is well established that diabetics are likely to develop cardiovascular diseases such as hypertension, atherosclerosis, stroke and congestive heart failure (Resnick & Howard, 2002). Furthermore, it is known that cardiovascular autonomic neuropathy is common in diabetics and contributes to the overall clinical pathology (Jermendy, 2003;Ewing *et al.*, 1980).

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In the 1920's, Walter Cannon, the patriarch of physiological sciences, divided the autonomic nervous system into the sympathetic and parasympathetic divisions. He proposed that the two systems antagonize each other in order to maintain "homeostasis", a word that he coined (Cannon, 1939). The concept of altered autonomic function as a pathophysiological condition dates from the 1920's with a report from Bradbury and Eggleston that suggested a neurogenic cause for postural hypotension (Bradbury & Eggleston, 1925). In more general terms, dysautonomia refers to a condition in which altered autonomic function adversely affects health. These conditions range from transient episodes in otherwise healthy individuals to progressive neurodegenerative diseases (Goldstein *et al.*, 2002).

Clinical studies have demonstrated that chronic diabetic complications occur late after disease onset, reflecting structural abnormalities in nerves, kidney, retina and blood vessels with the appearance strongly correlated with the duration of the diabetes and the level of glycemic control (1993;1998;Resnick & Howard, 2002). Autonomic neuropathy is a frequent complication of diabetes which is associated with high morbidity and mortality (Ewing *et al.*, 1980;Kempler *et al.*, 2002). The reported prevalence of diabetic autonomic neuropathy varies widely depending on the population studied and the methods of assessment. There are estimates that a large proportion of diabetics (up to 50%) show evidence of autonomic neuropathy (Kempler *et al.*, 2002;Maser *et al.*, 2003). Diabetic autonomic neuropathy typically occurs as a system-wide disorder affecting all parts of the autonomic nervous system (Vinik *et al.*, 2003). The neuropathology is usually manifest first in the longer autonomic nerves such as the vagus which accounts for almost 75% of parasympathetic activity (Ziegler, 1999). It is for this reason that even early onset effects of diabetic autonomic neuropathy are seen system wide. Vagal impairment seen in early diabetes leads to a relative predominance of sympathetic activity in sympatho-vagal balance. The increased mortality in diabetics is likely related to disorders in cardiovascular control, including impairment of autonomic reflexes leading to orthostatic hypotension, exercise intolerance, non-symptomatic myocardial infarction and sudden death from cardiac arrhythmias (Page & Watkins, 1978;Hilsted *et al.*, 1982). Meta analysis on the relationship between cardiovascular autonomic neuropathy and risk of mortality in diabetics showed an increase in diabetics with autonomic dysfunction (Maser *et al.*, 2003). The average mortality rate for ten studies over variable time spans was 29% in diabetics with autonomic dysfunction.

It should be emphasized that blood pressure and heart rate are not direct correlates of autonomic neural function. For evaluation of diabetic/autonomic interactions, it is necessary to examine the influence of the disease on heart rate and blood pressure variability, response to autonomic pharmacological blockade, functional changes in baro-and chemo reflexes and neuroanatomical changes. Autoregressive spectral analysis is a non-invasive, statistical means for studying autonomic function in both humans and animals (Malliani *et al.*, 1991;Joaquim *et al.*, 2004;Farah *et al.*, 2004). Heart rate variability (HRV) and blood pressure variability (BPV), estimated in time or frequency (spectral analysis) domain, are used to detect early abnormalities in autonomic modulation of the cardiovascular system (Pagani *et al.*, 1988;Spallone & Menzinger, 1997). Changes often occur before or without alterations in blood pressure and heart rate. For example, in human diabetics spectral analysis revealed a significant decrease in HRV, which was easily apparent before changes in other cardiovascular parameters (Pagani *et al.*, 1988). There is also an association between reduced HRV and increased risk for sudden death in patients with chronic heart failure (La Rovere *et al.*, 2003) or diabetes (Kataoka *et al.*, 2004). The importance of BPV in clinical pathologies was established with reports that increased BPV was associated with end-organ damage (Parati *et al.*, 1987;Zanchetti & Mancia, 1987).

In type 1 diabetes, there is evidence for early impairment of parasympathetic function, as evaluated by spectral analysis (Weston *et al.*, 1998;Poulsen *et al.*, 1997) or other cardiovascular

function tests (Weinrauch *et al.*, 1998). Type 1 diabetics also show high levels of plasma epinephrine and increased cardiac flow velocity, both signs of sympathetic activation (Ferraro *et al.*, 1990). In type 2 diabetes, glucose intolerance is the first step followed by insulin resistance and a resultant hyperinsulinemia (van Haeften *et al.*, 2002). It is thought that insulin and glucose activate the sympathetic nervous system with a consequent hyperdynamic state (increased heart rate and cardiac output), increased peripheral resistance, sodium retention and eventual development of hypertension (Facchini *et al.*, 1996;Lembo *et al.*, 1992;Anderson *et al.*, 1991). The consensus is that diabetic autonomic neuropathy is associated with parasympathetic withdrawal, sympathetic dominance and high blood pressure

Composite clinical results suggest that autonomic dysfunction is a common characteristic of both type 1 and type 2 diabetes. This neural disorder is of prime importance in the development of a variety of cardiovascular pathologies from atherosclerosis to myocardial infarction. This is emphasized in a recent publication by the American Diabetes Association for healthcare professionals that highlights the significance of diabetic neuropathy in disease evaluation and therapy (Boulton *et al.*, 2005). The objective of this review is to outline and discuss the animal models used for the study of insulin deficiency and insulin resistance with a focus on autonomic neural interactions. The ultimate goal is to understand the clinical relevance of cardiovascular autonomic dysfunction associated with diabetes.

2. INSULIN DEFICIENT MODELS

2.1 Streptozotocin induced diabetes

Experimental diabetes induced by streptozotocin (STZ) has been used extensively to study the relationship between diabetes and autonomic cardiovascular dysfunction. STZ destroys pancreatic β cells, resulting in a diabetic syndrome in animals, similar to that seen in human type 1 diabetes, characterized by hyperglycemia, hypoinsulinemia, glucosuria and loss in body weight (Junod *et al.*, 1969;Tomlinson *et al.*, 1992). With regard to cardiovascular parameters, STZ diabetic rats showed either no change or a reduction in blood pressure (Maeda *et al.*, 1995a;Maeda *et al.*, 1995b; De Angelis *et al.*, 2000;Dall'Ago *et al.*, 1997;Chang & Lund, 1986;Tomlinson *et al.*, 1990;Harthmann *et al.*, 2007;Souza *et al.*, 2007). In mice, STZ diabetes was associated with a mild hypertension which was corrected by treatment with angiotensin converting enzyme (ACE) inhibitors (Katoh *et al.*, 2000;Wichi *et al.*, 2007). With regard to heart rate, a resting bradycardia was observed in STZ diabetic rats, while in mice there were no changes (Maeda *et al.*, 1995a;De Angelis *et al.*, 2000;DeAngelis *et al.*, 2002;Senges *et al.*, 1980;Chang & Lund, 1986;Tomlinson *et al.*, 1990;Harthmann *et al.*, 2007;Souza *et al.*, 2007;Wichi *et al.*, 2007;Gurley *et al.*, 2006).

The arterial baroreflex system is a key mechanism for rapid adjustments in blood pressure. Indeed, the minimization of blood pressure variability by baroreflexes is important in maintenance of physiological homeostasis. Reduced baroreflex activity is an independent risk factor for sudden death after myocardial infarction (La Rovere *et al.*, 1998). Diabetic patients with normal cardiovascular reflexes have a lower incidence of mortality than diabetics with abnormal reflex function (Ewing *et al.*, 1980). Since arterial baroreflexes influence both sympathetic and parasympathetic outflow, disorders in autonomic neuronal systems (efferent or afferent) could have important consequences for cardiovascular health. The mechanisms of reflex dysfunction in diabetes have been widely investigated using animal models of insulin deficiency. In STZ diabetic rats, there was a reduction in cardiac vagal tone in the face of unaltered sympathetic tone. (Maeda *et al.*, 1995a;De Angelis *et al.*, 2000;Souza *et al.*, 2007). This was consistent with previous results which showed cardiac vagal neuropathy in rats with STZ diabetes (Wegner *et al.*, 1987). The impairment in baroreflex-mediated tachycardia occurred early in the time course of STZ diabetes (Page & Watkins, 1978;Maeda *et al.*, 1995a). In the chronic disease state, there was a reduction in both baroreflex-mediated

bradycardia and tachycardia, such that both sides of the reflex were dysfunctional (Tomlinson *et al.*, 1992;Harthmann *et al.*, 2007;Souza *et al.*, 2007). STZ diabetes in the spontaneously hypertensive rat (SHR) was associated with baroreflex impairment without changes in HRV (Farah *et al.*, 2007a). There are indications that maternal diabetes has prominent influences on baroreflex function and blood pressure in the offspring (Wichi *et al.*, 2005).

Arterial chemoreceptors are an important, but less studied, subset of afferents that participate in the control of cardiovascular autonomic function. In STZ diabetic rats, chemoreflex cardiovascular bradycardic and pressor responses to cyanide were impaired (Dall'Ago *et al.*, 1997). This impairment was sensitive to exercise intervention since aerobic training improved baro- and chemoreflexes as well as cardiac function in STZ diabetic rats (De Angelis *et al.*, 2000;Harthmann *et al.*, 2007;Souza *et al.*, 2007). This reinforces the idea that exercise can be used as a non-pharmacological approach to improve cardiovascular autonomic function in diabetes. Cardiopulmonary receptors are another important set of afferents that contribute to cardiovascular autonomic and volume control and are impaired in STZ diabetes. Serotonin or volume expansion are used to test cardiopulmonary reflexes resulting in bradycardia and hypotension. In STZ diabetic rats, the response to serotonin was unchanged while the response to volume expansion was enhanced (Oliveira *et al.*, 1999) (Patel, 1997).

The mechanisms for the changes in HRV associated with diabetes may be related to autonomic neuropathy. Indeed, HRV is used as a clinical marker for cardiac parasympathetic functional integrity (van Ravenswaaij-Arts *et al.*, 1993). Fazan and colleagues (Fazan, Jr. *et al.*, 1997) reported lowered HRV in STZ diabetic rats, associated with reductions in the low frequency (LF) and high frequency (HF) components. Measurement of the standard deviation of the adjacent pulse pressures, another index of HRV, also suggested alterations in autonomic function in STZ diabetic rats. Other studies showed a reduction in both HRV and BPV in STZ diabetic rats (Schaan *et al.*, 2004;Farah *et al.*, 2007a). The autonomic changes were correlated with plasma glucose, suggesting that increased glycemia was associated with neural dysfunction. Reinforcing this hypothesis, insulin replacement in STZ diabetic rats improved hemodynamic and autonomic status (Schaan *et al.*, 1997;Hicks *et al.*, 1998). In accordance with results in animal studies, clinical data showed autonomic nervous system abnormalities in diabetics (Kardelen *et al.*, 2006) that were improved with tight metabolic control (Hreidarsson, 1981;Ferreira *et al.*, 1998).

Autonomic cardiovascular analysis was also performed in STZ diabetic mice with results showing that neither heart rate or HRV were changed (Gurley *et al.*, 2006;Wichi *et al.*, 2007). These findings are in contrast to the decreased HRV seen in diabetic humans and rats. There was a reduction in BPV along with its low frequency spectral component in STZ diabetic mice (Wichi *et al.*, 2007), probably related to an enhancement in sympathetic modulation. Interestingly, the diabetes-induced reduction in BPV was absent in animals lacking angiotensin AT1a receptors (Wichi *et al.*, 2007). This may be explained by the fact that the autonomic nervous system is operating at maximal sympathetic status in the Ang AT1a gene deletion strain (Chen *et al.*, 2005).

The sympathetic and parasympathetic dysfunction observed in STZ model are correlated with biochemical and morphological changes in adrenergic and cholinergic systems. A reduction in dopamine β-hydroxylase activity was observed in the superior cervical ganglia (Schmidt & Plurad, 1986), while acetylcholinesterase levels were reduced in atria of STZ diabetic rats (Carrier & Aronstam, 1987). This increase may be related to the effective concentrations of acetylcholine acting on myocardial receptors, therefore, contributing to super sensitivity to muscarinic stimulation. Indeed, the bradycardiac response to muscarinic stimulation was enhanced in STZ diabetic rats (Dall'Ago *et al.*, 1997;Li *et al.*, 1989), suggesting receptor hypersensitivity linked to the characteristic reduction in parasympathetic activity seen in this

model. Additionally, atrial muscarinic receptors were increased in STZ-induced diabetic rats (Dall'Ago *et al.*, 2007). There was also a noticeable lack of atrial cholinergic terminals in the STZ diabetic animals, suggesting a level of cardiac parasympathetic denervation (Monckton & Pehowich, 1980;Schmidt *et al.*, 1983;Wright *et al.*, 2004). There is also data documenting neural degeneration after STZ treatment as seen by expression of genes linked to neural injury (Wright *et al.*, 2004). Functional defects in cardiac cholinergic nerves were also observed in diabetes induced by alloxan, a chemical toxin with similar diabetogenic effects as STZ (Kuntscherova & Vlk, 1970). In this model it was also reported atrial cholinergic and adrenergic super sensitivity associated with cardiac parasympathetic denervation with some indications of degeneration of sympathetic nerves (Tomlinson & Yusof, 1983).

2.2 Spontaneously diabetic Bio-Bred rats

A model for insulin deficient diabetes is the spontaneous diabetes which appears in Bio-Bred (BB/W) rats. The model shows autoimmune dysfunction with pancreatic atresia. There is an abrupt onset of symptoms, including weight loss, hypoinsulinemia, hyperglycemia and ketonuria (Crisa *et al.*, 1992). BB/W diabetic rats are normotensive, but show altered baroreflexes (Krizsan-Agbas & Bunag, 1991). There was no difference in the bradycardia elicited by phenylephrine; however, the reflex tachycardia elicited with nitroprusside was more pronounced. This suggests that baroreflexes are enhanced, specifically on the low pressure side (Krizsan-Agbas & Bunag, 1991). In terms of neuropathology, there is evidence for degenerative changes in both the sympathetic and parasympathetic systems (Addicks *et al.*, 1993;Yagihashi & Sima, 1986;Yagihashi & Sima, 1985). There is also data showing central sensory neuropathy, characterized by functional and structural changes in the optic nerve and retina (Sima *et al.*, 1992). There is little information on cardiovascular autonomic control or chemo, cardiopulmonary reflexes in this model.

2.3 Spontaneously Wistar Bonn/Kobori diabetic rats

A model for insulin deficient spontaneous diabetes is the *Wistar Bonn/Kobori* (WBN/Kob) rat. These animals develop hyperglycemia, glucosuria, hypoinsulinemia and glucose intolerance (Nakama *et al.*, 1985;Hashimoto *et al.*, 2001). The disease is prominent at 8–9 months and is seen only in males. Histopathological examination of the pancreas revealed fibrotic changes as early as 3 months of age. With advancing age, the fibrosis invades the islets, resulting in the clinical diabetic syndrome (Nakama *et al.*, 1985).

With regard to cardiovascular parameters, WBN/Kob rats (4–9 months) showed a reduction in heart rate without changes in blood pressure (Hashimoto *et al.*, 2001). Van Buren et al. (Van Buren *et al.*, 1998) suggested that the bradycardia was not related to autonomic dysfunction, since there was no evidence for changes in the parasympathetic nerves. However, WBN/Kob rats (4–5 months) lacked the circadian rhythm in heart rate variance, low to high frequency ratio (LF/HF), perhaps related to sympathetic overactivity (Hashimoto *et al.*, 2001). Thus, even at the pre-diabetic stage there is evidence for sympatho/vagal dysfunction.

As mature adults (9 months of age), this strain also shows an absence in a circadian rhythm for heart rate and blood pressure as observed in diabetic humans (Hashimoto *et al.*, 2001;Kardelen *et al.*, 2006;Nakano *et al.*, 1998;Hansen *et al.*, 1996;Berg *et al.*, 1997;Madacsy *et al.*, 1995). Power spectral analysis also indicated that both sympathetic and parasympathetic systems were dysfunctional (Hashimoto *et al.*, 2001). At this stage, there is insulin deficient diabetes, seen by marked hyperglycemia and hypoinsulinemia. Overall, the model is characterized by impairment of autonomic tone, consisting of sympathetic overactivity that precedes a reduction in parasympathetic activity. There is no information on the status of cardiovascular autonomic reflexes; however, there is data which shows sensory and motor neuropathies in the WBN/Kob model. Hearing impairment occurs with aging and is associated

with sensory nerve degeneration, indication of somatic neuropathy (Ishikawa *et al.*, 1995;Ishikawa *et al.*, 1997). Diabetic motor neuropathy was characterized by medullary sheath degeneration and demyelination, similar to changes observed in diabetic patients, but not seen in STZ diabetes (Ozaki *et al.*, 1996).

2.4 Non-obese diabetic (NOD) mice

The non-obese diabetic (NOD) mouse is a model of insulin deficiency, originally isolated as a spontaneous genetic mutation (Kikutani & Makino, 1992). Mice develop diabetes as the result of a band T-cell-mediated autoimmune attack on the pancreatic islets, a process which begins at approximately 4–8 weeks of age (Sreenan *et al.*, 1999). There is a greater incidence in females (80%) than males. NOD mice are hypoinsulinemic and hyperglycemic and require insulin replacement to survive. They have a short lifespan (5–8 weeks after onset of diabetic symptoms) and are difficult to study because of the rapid progression of the disease (Ordog *et al.*, 2000;Watkins *et al.*, 2000; Spangeus *et al.*, 2000). NOD mice also show early onset and marked neuropathy in the prevertebral sympathetic ganglia. A comparative study demonstrated that the severity of the autonomic damage is much greater in NOD mice than in STZ treated animals (Schmidt *et al.*, 2003). Cardiovascular characterization showed no change in blood pressure, a reduction in heart rate and enhanced baroreflex activity (Gross *et al.*, 2008). The study showed that the pathological changes were similar between the NOD model and human diabetics while the cardiovascular sequellae were different.

3. INSULIN RESISTANT AND OBESE MODELS

Since type 2 diabetes is the most common form in the human population, more than 90% of cases, availability of animal models for the disease is critically important. The mechanisms underlying the metabolic diseases and their associated cardiovascular pathologies has begun to be elucidated through the use of obese, diabetic animal models, such as the obese Zucker rat and *db*/*db* and *ob*/*ob* mice. They are widely used for experimental studies since they provide animal models which approximate the human condition in which obesity is often coupled with type 2 diabetes.

3.1 Obese Zucker rats

The Zucker strain, first described in the 1960's (Zucker, 1965), has a mutation in the leptin receptor gene resulting in impairment in the ability of leptin to suppress food intake. The Zucker rat phenotype is characterized by a variety of behavioral, neural, endocrine and metabolic disturbances that become apparent soon after weaning. These abnormalities include hyperinsulinemia, dyslipidemia, hyperphagia, hypothermia, increased sympathetic activity and reduced energy expenditure (Zucker, 1965;Cunningham *et al.*, 1986;Bray & York, 1979;York *et al.*, 1985). There are contradictory results as to the nature of the cardiovascular and autonomic characteristics of the Zucker model. While most reports show hypertension and enhanced sympathetic activity (Alonso-Galicia *et al.*, 1996;Carlson *et al.*, 2000;Kurtz *et al.*, 1989;Turner *et al.*, 1995;Gerges *et al.*, 2002;Schreihofer *et al.*, 2005;Stepp & Frisbee, 2002) there are others which indicate normal blood pressure and low sympathetic activity (Pamidimukkala & Jandhyala, 1996;Pawloski *et al.*, 1992;Levin *et al.*, 1983).

Cardiovascular function has been widely investigated in the Zucker model (Carlson *et al.*, 2000;Corry & Tuck, 1996;Ferrannini *et al.*, 1987). With regard to neural control, Ruggeri and colleagues (Ruggeri *et al.*, 2006) reported an enhancement in somato-sympathetic reflexes in insulin resistant, obese Zucker rats. In this study the animals were normotensive, suggesting that enhanced sympathetic reflex reactivity and insulin-resistance are seen before the onset of hypertension. Direct blood pressure measurements were made in conscious animals and results showed that the animals were not consistently hypertensive (Pamidimukkala & Jandhyala,

1996). For long term blood pressure measurements, radiotelemetry was used to compare diabetic obese and nondiabetic lean Zucker rats (12 weeks of age)(Carlson *et al.*, 2000). Diabetic obese rats showed a mild hypertension (increase of ~ 13 mmHg) and ganglionic blockade caused a greater decrease in blood pressure, suggesting enhanced sympathetic tone. Vasodilatory function (evaluated using acetylcholine) was significantly less in the Zucker rats while vasoconstrictor function was enhanced (Stepp & Frisbee, 2002).

Baroreflexes were blunted in anesthetized obese Zucker rats which could contribute to blood pressure elevation (Bunag *et al.*, 1990;Schreihofer *et al.*, 2007;Pamidimukkala & Jandhyala, 1996). Spectral analytical methods were used to evaluate autonomic cardiac modulation in obese and lean Zucker rats using telemetric monitoring of heart rate (Towa *et al.*, 2004). Heart rate was reduced in obese as compared to lean Zucker rats throughout the 24 h period with a diurnal variation in both strains. Cardiac HF power, LF power and LF/HF ratio were similar between obese and lean Zucker rats and the circadian rhythm of these parameters was largely preserved. Furthermore, autonomic pharmacological blockade (atropine and propranolol) produced similar effects on heart rate in the groups. The authors concluded that in conscious animals autonomic neural control of heart rate is not different in the Zucker model of obese diabetes. There is no data on chemo or cardiopulmonary reflexes or BPV in the obese Zucker model.

3.2 Genetic diabetic db/db mice

The genetically diabetic *db/db* mouse (BKS.Cgm+/+Leprdb/j) has a leptin mutation resulting in hyperglycemia, insulin resistance and hyperinsulinemia (Chen *et al.*, 1996). The *db*/*db* is widely used as a model of insulin resistant type 2 diabetes. There is evidence for increased *in vitro* vascular contractility in this model (Kanie & Kamata, 2000) with less information on *in vivo* cardiovascular changes. Using the tail cuff method for measuring blood pressure, results were inconsistent with data showing no change, increase or decrease in blood pressure in *db/db* mice (Bagi *et al.*, 2005;Brezniceanu *et al.*, 2008;Kosugi *et al.*, 2006). In order to accurately characterize the cardiovascular phenotype in the *db/db*, a developmental time course was conducted using radiotelemetry (Senador *et al.*, 2007). Early in the disease process (8–9 weeks), there were no differences in blood pressure between diabetics and controls. However, with aging (14–15 weeks) and decreasing insulin levels, blood pressure was increased. There was a consistent bradycardia at both ages. With regard to autonomic neural changes, there was evidence for peripheral neuropathy and reduction in norepinephrine levels in heart, kidney and salivary glands (Giachetti, 1978).

Noradrenergic responsiveness of the heart was reduced and there were indications of sympathetic denervation (Tessari *et al.*, 1988). Spectral methods were used for evaluation of autonomic function and there was no change in HRV, BPV or baroreflex function (Senador *et al.*, 2007). Findings are not supportive of major functional autonomic changes.

3.3 Genetic obese KKAy and ob/ob mice

Genetic obese KKAy and *ob/ob* mice are obese models used in the study of diabetic complications. KKAy is a congenital strain established by the transduction of the yellow obese gene (Ay) into the moderate hyperglycemic KK strain (Iwatsuka *et al.*, 1970). KKAy mice are obese with high blood pressure, increased urinary catecholamines and exacerbated responses to sympathetic blockade, suggesting a sympathetic role in the hypertension (Aizawa-Abe *et al.*, 2000;Ohashi *et al.*, 2006). The *ob/ob* strain has a mutation in the ob gene, resulting in leptin deficiency (Zhang *et al.*, 1994). Short term direct blood pressure measurements (Mark *et al.*, 1999) suggested that the *ob/ob* mice are hypotensive with low sympathetic nerve activity (Young & Landsberg, 1983). In contrast, when blood pressure was measured chronically with radiotelemetry, ob/ob mice were hypertensive during the light period (Swoap, 2001). There

are no specific studies on autonomic control of the circulation in these genetically modified models.

4. DIETARY MODELS

There is much interest in the use of dietary methods for the induction of diabetic states, particularly because of the relevance to the human condition. Cardiovascular dysfunction is associated with obesity and metabolic disorders which occur when animals are fed a high fructose or fat diet. The rational for the use of high fructose as a test diet comes from the predominance of high fructose corn syrup in processed food and increased consumption. A diet high in fructose may lead to insulin resistance, obesity, hypertension and lipid abnormalities, symptoms associated with type 2 diabetes (Basciano *et al.*, 2005). Fructose-fed rats show a moderate hypertension and glucose intolerance, associated with high levels of plasma insulin, cholesterol and triglycerides (Hsieh, 2005;Katovich *et al.*, 2001;Kamide *et al.*, 2002;Dai & McNeill, 1995;Dai *et al.*, 1994). Additionally, in fructose fed rats there was a parasympathetic impairment that was positively correlated with insulin resistance (Brito *et al.*, 2008). In mice, there is evidence that a fructose diet alters glucose metabolism and lipid levels (Nagata *et al.*, 2004;Luo *et al.*, 1998). Chronic telemetric recording of blood pressure in mice showed that fructose increased blood pressure as well as BPV (Farah *et al.*, 2006). The changes were correlated with the light/dark cycle with the highest blood pressure and BPV observed during the dark phase. This is an important consideration since this is the time when mice are active (grooming, eating and drinking) and the time when sympathetic activity should be highest. Our results are in accordance with clinical studies which show that diabetic hypertensive patients presented an increase in BPV (Mancia *et al.*, 1983). Variability changes associated with hypertension may contribute to the cardiovascular risks related to high fructose consumption.

In terms of the mechanisms behind the fructose-induced cardiovascular changes, there is evidence for a role of the sympathetic nervous and renin angiotensin systems (RAS). Sympathectomy (adrenal medullectomy coupled with neurotoxin exposure) attenuated the development of hypertension in rats fed a high fructose diet, suggesting a role for the sympathetic nervous system (Verma *et al.*, 1999). Fructose feeding also increased plasma and urinary catecholamines and adrenergic receptor expression (Kamide *et al.*, 2002;Dai *et al.*, 1994). Evidence for a role for the renin angiotensin system in fructose-induced cardiovascular changes was seen by the increased expression of Ang receptors in the vasculature and depressor effect of angiotensin receptor antagonists (Hsieh, 2005;Katovich *et al.*, 2001). In mice, there is data which shows that a high fructose diet caused activation of the vascular and brain renin angiotensin system (Shinozaki *et al.*, 2004;Farah *et al.*, 2006). Ang AT1a knock out (KO) mice were used to determine the role of Ang signaling in the mediation of the dietary fructose induced changes in autonomic and metabolic parameters (Farah *et al.*, 2007b). A key finding was that the fructose-induced blood pressure increase and the vascular autonomic responses were absent in the AT1aKO, indicating interactions between the Ang and autonomic systems. Likewise there were differences in the fructose induced changes in glucose tolerance, with a greater inhibition of glucose tolerance in the control animals.

There is much evidence showing an association between obesity, hypertension and diabetes. In animal studies this is produced by consumption of a high fat diet (called the Western diet) which causes hypertension, lipid abnormalities and arterial hypertrophy (Dobrian *et al.*, 2000;Boustany *et al.*, 2004;Bunag *et al.*, 1990) Reflex tachycardia during depressor responses was unchanged in obese rats while reflex bradycardia during pressor responses was impaired (Bunag *et al.*, 1990). Studies using pharmacological blockade suggested that parasympathetic input was reduced in the obese rat model. Mice (C57BL6) fed a high fat/high carbohydrate diet develop a form of non insulin dependent diabetes as seen by the high glucose and insulin

(Surwit *et al.*, 1988). These animals also show a moderate hypertension, no change in heart rate and an increase in HRV (Williams *et al.*, 2003;Rahmouni *et al.*, 2005) as well as an accentuated adrenergic response to stress (Surwit *et al.*, 1988). An attractive mechanism by which type 2 diabetes associated hypertension could develop in C57BL6 mice is via a genetic alteration that enhances the sympathetic activation associated with the high fat carbohydrate feeding (Landsberg & Young, 1978). The association of an increased blood pressure rather than heart rate response to ganglionic blockade suggests that an excessive recruitment of sympathetic outflow to target organs is an important characteristic of this form of diet induced diabetes/hypertension. Sympathetic overactivity could be a hypertensive as well as a diabetogenic mechanism.

5. CONCLUSIONS

There is consistent evidence that the autonomic nervous system plays a key role in diabetic pathophysiology in humans and in animal models. Among the different neuropathies, cardiovascular autonomic dysfunction, associated with poor prognosis and increase in morbidity and mortality, has been a consistent focus of study. The study of blood pressure and heart rate alterations, as well as the autonomic control of circulation impairment using physiological, pharmacological, anatomical and molecular methodologies in experimental animal models of insulin deficiency and resistance has contributed to our knowledge of diabetic autonomic dysfunction. New approaches, such as genetically modified models, can provide mechanistic information on the genetic and environmental factors involved in the development of diabetic dysautonomia. The evaluation of data obtained from both clinical and experimental models allows for a better understanding of the etiology of the disease as related to the autonomic nervous system.

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