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Extended longitudinal analysis of arterial pressure and heart rate control in unanesthetized rats with type 1 diabetes

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ABSTRACT

We recorded arterial pressure (BP) and heart rate (HR) in type-1 diabetic rats vs. controls for >6 months. Diabetic rats (DIAB) were maintained on insulin from the day glucose >250 mg/dl ("Day 0"). Weight was similar between groups until ~3 weeks before Day 0 when the weight in DIAB transiently lagged the controls (CONT); this difference was maintained throughout the study, but both groups otherwise gained weight in parallel. Plasma glucose attained 371 ± 109 (SD) mg/dl by day 1 in DIAB. Mean BP was similar across groups, and declined through the initial 4–6 months in both the CONT (at −0.06 ± 0.04 mm Hg/day) and in the DIAB (at −0.14 ± 0.21 mm Hg/day; NS vs. CONT). HR in the CONT (Month 1: 341 ± 13 bpm) exceeded DIAB (325 ± 25 bpm) through ~6 months after Day 0, and also decreased progressively over this period in CONT (−0.19 ± 0.14 bpm/day) and DIAB (−0.29 ± 0.23 bpm/day; NS vs. CONT) before leveling. The BP power within 0.35–0.45 Hz changed during the 90 min before vs. after the transition from dark to light, and light to dark; there were no between group differences. The slope of the log–log linear portion of the BP power spectrum between 1.0/h and 1/min was similar across groups, and increased in both from month 1 to month 6. Regulatory mechanisms maintain similar profiles in BP and HR in diabetic vs. control animals through the initial half year of the disease.

1. Introduction

Diabetes and its complications are known to affect the autonomic control of cardiovascular, neural, renal, gastrointestinal, sexual and other functions (reviewed in American Diabetes Association and American Academy of Neurology, 1988; Vinik et al., 2003; Vinik and Ziegler, 2007; Freeman, 2005), and diabetic cardiovascular autonomic neuropathy is "one of the most overlooked of all serious complications of diabetes…" (Vinik and Ziegler, 2007). The disease may damage small fibers, large fibers or both; the sympathetic and parasympathetic systems may each be affected by the disease process (Hosking et al., 1978; Hilsted, 1982; Pfeifer et al., 1982). Autonomic neuropathy (AN) causes widespread disturbances in visceral function, particularly including that of the heart and blood vessels. A study of 605 patients followed for 9 years revealed that individuals with diabetes and low autonomic function had approximately a doubled risk of mortality (Gerritsen et al., 2001). Finally, hypertension is common in diabetic patients (e.g.., Czupryniak et al., 2006). Even if it is granted that in many cases cardiovascular disease co-exists with diabetes, it is difficult to demonstrate from observations in humans any causal relationships between diabetes and associated cardiovascular co-morbidities. It is clear, however, that knowledge of arterial pressure *per se* is important at all developmental stages in diabetes (ACCORD Study Group, 2010).

Many previous animal studies in experimental diabetes used alloxan or streptozotocin administration to kill the insulin-secreting beta cells of the subject's pancreas. Alternatively, the Bio-Breeding Labs bred the BBDP/Wor rat, or 'BB' rat, which spontaneously develops an autoimmune, insulin-dependent diabetes that resembles the human disease in many ways. In particular, the pancreas of the affected BB animals shows lymphocytic insulitis, fibrosis and the absence of beta cells (Chappel and Chappel, 1983). Autonomic and sensory neuropathies have been described in the BB rat. Alterations in nerve collagens can be detected by 4 months (Wang et al., 2003). Nerve conduction velocity slows

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progressively, declining by 17% at 14 months (Sima et al., 2000). At 4 and 8 months the BBDP/Wor shows a progressive redistribution of nodal Na^+ channels across the paranodal and internodal regions that are associated with the conduction slowing (Cherian et al., 1996). Axonal dystrophic changes in sympathetic fibers, which are the hallmark of AN in human diabetics, are consistently evident in the BB rat after ~8 months of diabetes (Schmidt et al., 2003; Yagihashi and Sima, 1985a,b). The main structural abnormality consists of expanded axons containing a variety of normal and abnormal subcellular structures, prompting the investigators to conclude that "dystrophic and degenerative axonopathy is a reproducible structural hallmark of diabetic sympathetic neuropathy" in the BB rat (Yagihashi and Sima, 1985b). BB rats diabetic for 28 weeks have a 57% loss relative to controls in ventricular myocardial sympathetic nerve fibers and varicosities and, in atrial tissue, a 42% loss in sympathetic axon profiles containing varicosities (Addicks et al., 1993).

The cardiovascular consequences in the BB rat of the degenerative changes in autonomic nerves that occur over time are of obvious interest. Multiple standard analyses, such as comparison of absolute mean values across groups, are insightful, but power spectral analysis of a given variable quantifies the dynamics of that 'signal', and helps delineate those regulatory mechanisms responsible for a given fluctuation of the variable. For example, in dog heart rate (HR) changes that characteristically repetitiously recur with a period of >25 s in the resting animal appear as a concentration of power in the computed HR power spectrum within the range of ~0.003 to 0.09 Hz (i.e., averaging ~1 cycle per 50 s or 0.02 Hz). The power within the HR spectrum resultant from this recurring 'periodicity', classically attributed to vasomotor activity (e.g., Akselrod et al., 1981), is significantly attenuated, but not eliminated, by selectively surgically eliminating the parasympathetic innervation of the SA-node, while leaving sympathetic innervations intact; it is further decreased by the addition of pharmacologic β-adrenergic blockade (Randall et al., 1991). Conversely, the peak in the HR power spectrum at ~0.32 Hz, that is tightly linked to respiration in the resting dog, is virtually eliminated by that same selective SA-nodal parasympathectomy (Randall et al., 1991). More recent recordings of sympathetic nervous activity (SNA) and changes in arterial blood pressure (BP) in unanesthetized rat revealed concentrations of power in both spectra centered around ~0.4 Hz; these recordings further demonstrated that fluctuations in BP and SNA are tightly coupled, or 'coherent', at this frequency (Brown et al., 1994; Burgess et al., 1999). This portion of the 'mid-frequency' spectrum represents primarily, though not exclusively, harmonic power that can be modeled as resultant from the natural frequency of the baroreflex (e.g., Burgess et al., 1997; Cerutti et al., 1994). These observations suggest that assessment of differences in the BP power spectra of diabetic vs. age-matched control rats centered within the mid-frequency range would be informative. Sanyal et al., 2002 and Zhang et al., 1990 have, in fact, examined other aspects of BP variability in the BB rat associated with autonomic function. Conversely, no one has described how mid-frequency rhythm may change across a 24-hour, light:dark cycle. That is, the 'dynamics' of the 0.4 Hz rhythm itself have not been examined.

One additional feature of the BP power spectrum is noteworthy: the log of BP spectral power increases linearly as the log of frequency decreases. This inverse 'log–log linear' behavior is seen widely in nature (e.g., Voss, 1989) and, more particularly, is evident in HR and BP spectra (e.g., Butler et al., 1994). This so-called '$1/f$' (or $1/f^{\beta}$, see below) character is indicative of 'fractal' or 'self similar' behavior. That is, the statistical characteristics of a signal possessing self-similarity look remarkably similar irrespective of the 'scale' being examined: the undulations of a coastline look comparable when examined across meters, kilometers or hundreds of kilometers. With respect to biological signals that display this phenomenon, the persistent and beguiling tenet is that such remarkable behavior must tell us something truly fundamental about the forces that shape that variable's behavior, though, admittedly, this ultimate 'meaning' remains elusive. The use of BP telemetry would now allow the extended recording of arterial pressure required for such broad frequency measurements in diabetic vs. non-diabetic rat. In sum, frequency domain analyses provide useful means to quantify BP 'dynamics' and point towards the underlying autonomic control of those rhythms.

The purposes of this study were, first, to document changes in weight, plasma glucose, mean arterial BP (mBP) and HR in diabetic animals (DIAB) and age-matched controls (CONT) during the months following the former animals' becoming type 1 diabetic. We tracked arterial pressure and HR extending up to 6 months or more to determine if there were differences between the two groups which might be attributable to the diabetic state and/or dysautonomia. Second, we also quantified any differences between the two groups in the dynamics of the harmonic variability of mBP and of its fractal power. In particular, we documented any differences in mBP power within the range 0.35–0.45 Hz between DIAB and CONT, any changes in such power across a 24 hour period, and the value of β computed between 1.0/h and 1/min. Finally, we interpreted our data in so far as possible in terms of the effects of dysautonomia known to accompany extended periods of diabetes in the BBWP/Wor rat. A preliminary report of these findings has been published (Anigbogu et al., 2005).

2. Materials and methods

2.1. Animals

A total of 34 diabetes prone (BBDP/Wor) and 26 diabetes resistant (control; BBDR/Wor) rats participated in one or more stages of this study. Three additional diabetes prone animals never became diabetic; data from these latter animals are not included in the analyses reported here. All animals were obtained from the Biomedical Models Inc. (Rutland MA). The diabetes prone rats spontaneously develop an autoimmune, abrupt onset type 1 diabetes mellitus between 50 and 120 days of age characterized by polydipsia, polyuria and hyperglycemia (Chappel and Chappel, 1983). The rats were obtained from the vendor at 31–45 days of age. The animals were housed in an isolated, sound shielded, limited access room where the temperature was controlled at 72 °F, 56% humidity, and a 12/12 hour light/dark cycle. The rats were fed on standard rat chow (Harlan Teklad 2018, Madison WI) and had access to water *ad-libitum*. The study was approved by the University of Kentucky Animal Care and Use Committee.

The diabetes prone animals were weighed each morning, including weekends, and blood glucose was measured in a drop of blood from the saphenous vein using the One-Touch Ultra glucometer (LifeScan Inc., Milpitas CA). The control animals were weighed each Friday, and their plasma glucose determined, also from prick of the saphenous vein.

2.2. Surgery

All surgery was performed under anesthesia (sodium pentobarbital; 65 mg/kg, IP) with procedures appropriate for rodent survival surgery. Surgery was performed prior to the diabetes prone animals becoming diabetic, and at a similar age in the matched-controls. The abdominal aorta was accessed via a laparotomy. The sensory element of a Data Sciences International (DSI) probe (model TA11PA-C40) was placed into the aorta in 12 diabetes prone and 12 control rats via a puncture. The probe's 'catheter' was secured in place with surgical glue. The body of the probe that contains the sensor, transmitter and battery was sutured to the interior abdominal wall. The incision was closed and the rat monitored until it aroused from the anesthetic. The animal was returned to the home cage once it had aroused and was self grooming.

2.3. Experimental protocol

The day the animal first showed a morning blood glucose level above 250 mg/dl was taken as onset of diabetes mellitus and designated "Day