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FUNCTIONAL CHANGES IN BARORECEPTOR AFFERENT, CENTRAL AND EFFERENT COMPONENTS OF THE BAROREFLEX CIRCUITRY IN TYPE 1 DIABETIC MICE (OVE26)

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Abstract—Baroreflex control of heart rate (HR) is impaired in diabetes mellitus. We hypothesized that diabetes mellitus induced functional changes of neural components at multiple sites within the baroreflex arc. Type 1 diabetic mice (OVE26) and FVB control mice were anesthetized. Baroreflex-mediated HR responses to sodium nitroprusside- (SNP) and phenylephrine- (PE) induced mean arterial blood pressure (MAP) changes were measured. Baroreceptor function was characterized by measuring the percent (%) change of baseline integrated aortic depressor nerve activity (Int ADNA) in response to SNP- and PE-induced MAP changes. The HR responses to electrical stimulation of the left aortic depressor nerve (ADN) and the right vagus nerve were assessed. Compared with FVB control mice, we found in OVE26 mice that (1) baroreflex-mediated bradycardia and tachycardia were significantly reduced. (2) The baroreceptor afferent function in response to MAP increase did not differ, as assessed by the parameters of the logistic function curve. But, the inhibition of Int ADNA in response to MAP decrease was significantly attenuated. (3) The maximum amplitude of bradycardic responses to right vagal efferent stimulation was augmented. (4) In contrast, the maximum amplitude of bradycardic responses to left ADN stimulation was decreased. Since Int ADNA was preserved in response to MAP increase and HR responses to vagal efferent stimulation were augmented, we conclude that a deficit of the central mediation of baroreflex HR contributes to the overall attenuation of baroreflex sensitivity in OVE26 mice. The successful conduction of physiological experiments on the ADN in OVE26 mice may provide a foundation for the understanding of cellular and molecular mechanisms of diabetes-induced cardiac neuropathy.

Key words: baroreflex, brainstem, baroreceptor, parasympathetic, diabetic neuropathy, OVE26.

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Abbreviations: ADN, aortic depressor nerve; ADNA, aortic depressor nerve activity; AP, arterial pressure; ECG, electrocardiogram; HR, heart rate; MAP, mean arterial pressure; OVE26, transgenic type 1 diabetic; PAP, phasic arterial pressure; PE, phenylephrine; SNP, sodium nitroprusside; STZ, streptozotocin.

Diabetes mellitus is commonly associated with cardiovascular diseases including hypertension, atherosclerosis, cardiomyopathy, congestive heart failure, and autonomic neuropathy (Liang et al., 2002; Cai and Kang, 2003; Ostergaard et al., 2005; El-Menyar, 2006; Movahed, 2007; Vinik and Ziegler, 2007). Of particular clinical and physiological importance is the baroreflex control of heart rate (HR) (De Angelis et al., 2002). In both diabetic patients and experimental diabetic animals, a rich body of literature has demonstrated that the baroreflex control of HR is significantly impaired (McDowell et al., 1994a,b; Salgado et al., 2001; De Angelis et al., 2002; Dall'Ago et al., 2007). The impairment in baroreflex control, in concert with other disease processes occurring in the diabetic heart, may likely contribute to life-threatening arrhythmias and even sudden death, such as "dead-in-bed syndrome" in young adults (Weston and Gill, 1999; Bell, 2006). The impaired baroreflex sensitivity has been widely used as an important risk factor for life-threatening arrhythmias (La Rovere et al., 1998; Johansson et al., 2007; Movahed, 2007). Unfortunately, our knowledge about the exact location(s) of diabetes-induced change in baroreflex sensitivity remains fragmentary. Therefore, a systematic study of diabetes-associated functional changes in the neural components at multiple sites within the baroreflex pathway is important.

The baroreflex pathway includes both parasympathetic and sympathetic components (Armour, 1999). In particular, the baroreflex arc contains baroreceptor afferents, parasympathetic efferents, cardiac ganglia, sympathetic efferents, and the brain areas which modulate autonomic function (Loewy and Spyer, 1990). Conceivably, multiple, non-mutually exclusive mechanisms could be proposed to explain the change of baroreflex-mediated HR responses in diabetes, including: (a) a change of baroreceptor afferent (Salgado et al., 2001; Fazan et al., 2006); (b) attenuation of vagal efferent control of HR (Van Buren et al., 1998); (c) a deficit in central mediation of baroreflex (McDowell et al., 1994a,b; De Angelis et al., 2002; Dall'Ago et al., 2007).

The current available data seem incomplete or controversial. For the baroreceptor afferent component, diabetes induces baroreceptor afferent nerves atrophy, but does not reduce baroreceptor function (Salgado et al., 2001; Fazan et al., 2006). Therefore, the anatomical data could not explain the dysfunction in baroreflex control of HR. For the parasympathetic efferent component, HR responses to electrical stimulation of the vagus nerve were found to be either attenuated, unchanged, or enhanced by diabetes (McDowell et al., 1994b; Van Buren et al., 1998; De An-

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gelis et al., 2002; Dall'Ago et al., 2007). For the central component, McDowell et al. (1994a) and Dall'Ago et al. (2007) suggested that a deficit might have occurred in the central autonomic nervous system in experimental diabetic models. However, a direct test of the central mediation for baroreflex control of HR in diabetes had not yet been done. In the present study, we aimed to determine which neural components within the baroreflex circuitry could be changed by diabetes. We hypothesized that diabetes would attenuate the functions of baroreceptor afferent, central, and parasympathetic efferent components within the baroreflex circuitry.

Within the baroreflex circuitry, the parasympathetic system was considered to be the major pathway in baroreflex-mediated HR control (McDowell et al., 1994a; De Paula et al., 1999; Salgado et al., 2007). Consistent with this, we recently demonstrated that extensive lesion of vagal motoneurons in the nucleus ambiguous (NA) with domoic acid almost completely eliminated the baroreflex control of HR (Cheng et al., 2004), implying that vagal cardiac motoneurons are one of the major elements within the baroreflex circuitry, and play a key role in baroreflex-mediated HR responses. Therefore, we focused on the parasympathetic nervous system in the present study.

The commonly-used experimental models for type 1 diabetes have been streptozotocin (STZ) or alloxan-induced type 1 diabetes in rats and rabbits (McDowell et al., 1994a,b; De Angelis et al., 2002). Despite the advances afforded by the study in these experiment models, similar studies have not been conducted in mice. In the present study, we used transgenic type 1 diabetic mice (OVE26) to study the functional changes of aortic baroreceptor afferent, vagal efferent, and central components. The OVE26 mouse is a widely used model for the study of cardiovascular complications and nephropathy in type 1 diabetes (Liang et al., 2002; Ye et al., 2003, 2004, 2005; Zheng et al., 2004; Shen et al., 2005, 2006). The use of diabetic mice would make investigation of the cellular and molecular mechanisms amenable to transgenic manipulations (Epstein et al., 1989, 1992; Ye et al., 2004, 2005; Shen et al., 2005, 2006).

EXPERIMENTAL PROCEDURES

FVB and OVE26 mice (age: 5–6 months) were used. OVE26-positive mice were recognized by the presence of small eyes caused by the cointegration of the GR19 gene (Epstein et al., 1989), which is expressed in the eye. All transgenic and nontransgenic animals were maintained on the inbred FVB background. Mice were maintained on a 12-h light/dark cycle and received food and water *ad libitum*. Procedures were approved by the University of Central Florida Animal Care and Use Committee, and were in agreement with the National Institutes of Health guide for the care and use of laboratory animals. Efforts were made to minimize animal suffering and the number used.

OVE26 mice develop type 1 diabetes because of beta cell specific damage due to a calmodulin transgene regulated by the insulin promoter (Epstein et al., 1989). The beta cell impairment in OVE26 mice results in sustained high blood glucose levels well over 500 mg/dl and reduced insulin secretion by 30 days of age. The advantages of this model for the study of diabetes-associated complications are straightforward: direct damage is limited to the beta cells, diabetes develops early, and very severe diabetes lasts for more than 1 year without any insulin treatment (Epstein et al., 1989, 1992; Zheng et al., 2004).

Surgical procedures

Mice were anesthetized initially by 3% isoflurane inhalation, or a ketamine (91 μg/g, i.p.)/acepromazine (1.8 μg/g, i.p.) cocktail.

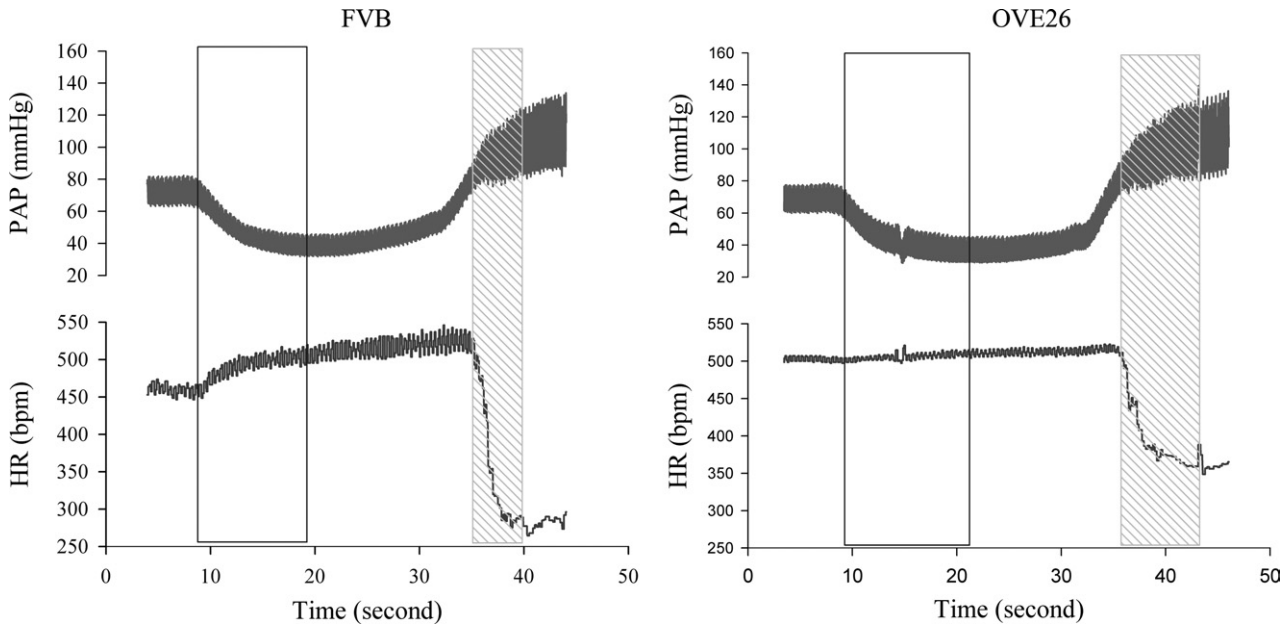

Fig. 1. Original recordings of HR responses to PAP changes induced by SNP/PE sequential administration from representative FVB and OVE26 mice. SNP/PE sequential administration included two phases: tachycardic and bradycardic responses. During SNP application (tachycardic phase), MAP and HR changes from the baseline of MAP to the nadir of MAP were measured as shown in the open box. During PE application (bradycardic phase), MAP and HR changes were measured from the peak of the HR to the nadir of the HR as shown in the hatched box.