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Impairment of baroreflex control of heart rate and structural changes of cardiac ganglia in conscious streptozotocin (STZ)-induced diabetic mice

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ABSTRACT

Baroreflex control of heart rate (HR) is impaired in human diabetes mellitus and in large experimental models. However, baroreflex impairment in diabetic mouse models and diabetes-induced remodeling of baroreflex circuitry are not well studied. We examined the impairment of baroreflex control of heart rate (HR) and assessed structural remodeling of cardiac ganglia in the streptozotocin (STZ)-induced diabetic mouse model. FVB mice were either injected with vehicle or STZ. Group 1: mice were anesthetized and the femoral artery and vein were catheterized at the 30th day after vehicle or STZ injection. On the second day after surgery, baroreflex-mediated HR responses to sodium nitroprusside (SNP) and phenylephrine (PE)-induced mean arterial blood pressure (MABP) changes were measured in conscious mice. Group 2: Fluoro-Gold was administered (i.p.) to label cardiac ganglia in each mouse at the 25th day after vehicle or STZ injection. After another five days, animals were perfused and cardiac ganglia were examined using confocal microscopy. Compared with control, we found in STZ mice: 1) the HR decreased, but MABP did not. 2) The PE-induced increases of MABP were decreased. 3) Baroreflex bradycardia was attenuated in the rapid MABP ascending phase but the steady-state ΔHR/ΔMABP was not different at all PE doses. 4) SNP-induced MABP decreases were not different. 5) Baroreflex tachycardia was attenuated. 6) The sizes of cardiac ganglia and ganglionic principal neurons were decreased. 7) The ratio of nucleus/cell body of cardiac ganglionic neurons was increased. We conclude that baroreflex control of HR is impaired in conscious STZ mice. In addition, diabetes may induce a significant structural remodeling of cardiac ganglia. Such an anatomical change of cardiac ganglia may provide new information for the understanding of diabetes-induced remodeling of the multiple components within the baroreflex circuitry.

1. Introduction

Baroreflex control of heart rate (HR) is impaired in diabetes mellitus (Vinik and Ziegler, 2007). Such impairment is often associated with life-threatening arrhythmias (El-Menyar, 2006; Lawrence et al., 1997). Consistent with symptoms observed in diabetic patients, baroreflex control of HR is significantly impaired in chemically-induced diabetic rabbits and rats (De Angelis et al., 2002; McDowell et al., 1994a,b; Salgado et al., 2001). Though some progress has been made toward the understanding of impaired baroreflex function and structural remodeling of aortic depressor nerves in the experimental diabetic rat model (Dall'ago et al., 2007; do Carmo et al., 2007; Fazan et al., 2006), baroreflex impairment and structural remodeling of different neural components within the baroreflex circuitry have not yet been systematically studied. The baroreflex circuitry includes both parasympathetic and sympathetic components. In particular, the baroreflex circuitry contains baroreceptor afferents, parasympathetic efferents, cardiac ganglia, sympathetic efferents, and the brain areas which modulate autonomic function (Loewy and Spyer, 1990). Conceivably, diabetes may induce structural and functional changes at multiple, non-mutually exclusive sites within the baroreflex arc. Our long-term goal is to systematically examine diabetes-induced alteration of the multiple neural components within the baroreflex circuitry. Previously, we demonstrated that parasympathetic cardiac motoneurons in the nucleus ambiguus in the brainstem project extensively to cardiac ganglia in rats and mice (Ai et al., 2007; Cheng and Powley, 2000; Cheng et al., 2004a). Lesions of this pathway almost completely abolished baroreflex control of HR (Cheng et al., 2004b). Therefore, the parasympathetic

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pathway plays a key role in baroreflex control of HR. Accumulating evidence suggests that cardiac ganglia, which have been traditionally viewed as simple relay stations for signals from the brain, may function as local integration centers to mediate the central control of the heart (Armour, 2007). Therefore, it is very important to study diabetes-induced anatomical changes of cardiac ganglia which are one of the key components in brain–heart connection.

Since mice are particularly useful mammalian models that are not only susceptible to different genetic manipulations but can also be used in physiological, anatomical, cellular, and molecular studies (Chapleau et al., 2001; Chapleau and Abboud, 2004), it is very important to characterize the functional and morphological changes of the baroreflex circuitry of diabetic mouse models. Recently, it was reported that despite pathomorphological similarities of the diabetic autonomic neuropathy between patients with diabetes and non-obese diabetic (NOD) mice (Schmidt et al., 2003), baroreflex sensitivity was higher in diabetic NOD mice than in controls (Gross et al., 2008). Therefore, the use of NOD mice as a functional model for human diabetes may be questionable (Gross et al., 2008) and other diabetic mouse models should be studied. In this study, we examined the impairment of baroreflex control of HR and structural remodeling of cardiac ganglia in streptozotocin (STZ)-induced diabetic mice. STZ-induced diabetic mice were chosen because they are an important and useful diabetic model. Previously, we also used a transgenic Type 1 diabetic mouse model (OVE26) (Gu et al., 2008, 2009; Lin et al., 2009; Li et al., in press). The OVE26 mouse was created in 1989 for the sole purpose of analyzing pancreatic beta cell function (Epstein et al., 1989). It was not until 2002 that the model was considered for the study of diabetic complications (Liang et al., 2002). Since then, more than two dozens of papers have been published using OVE26 diabetic mice to study diabetes-induced cardiac, renal, and autonomic complications of diabetes (e.g., Liang et al., 2002, Ye et al., 2003; Zheng et al., 2004, 2008; Lin et al., 2009). However, Since the OVE26 mouse model has just recently been used for the study of diabetes-induced neuropathy, it is very important to compare the data from STZ diabetic mice with OVE 26 diabetic mice. In our series of studies, the use of two different diabetic models rather than only one may provide information about whether the results obtained are due to the effects of diabetes or are specific to the model tested.

2. Materials and methods

FVB male mice (age: 4–5 months) were used. Previously, we have used OVE26 diabetic mice in several studies (Gu et al., 2008, 2009; Yan et al., 2009; Li et al., in press). The transgenic line of OVE26 diabetic mice was constructed on the inbred strain FVB. The FVB strain has been moderately popular for producing transgenic mice because it has easy to inject pronuclei at the single cell stage and it is a healthy, fertile breeder (Epstein et al., 1989, 1992). To make our present study compatible with our other studies, FVB male mice were used in this study. Female and male FVB mice were obtained from Jax Laboratory at age of 2–3 months. In each cage, one male mouse was housed with three female mice. All animals used in this study were from the first generation. Mice were maintained on a 12-h light/dark cycle and received food and water ad libitum. All animals were then maintained in the transgenic animal facility at the University of Central Florida. Procedures were approved by the University of Central Florida Animal Care and Use Committee and followed the guideline established by the NIH. Efforts were made to minimize the number of animals used.

2.1. Induction of diabetes using streptozotocin (STZ)

FVB male mice received three separate intraperitoneal (i.p.) injections of freshly-dissolved streptozotocin (STZ) in 0.01 M citrate buffer (pH 4.5) 0.1 mol/L sodium citrate, (pH 4.5) or vehicle (citrate buffer) over three subsequent days (0.07 mg/g/day). Fasting blood glucose levels (measured after 12 h of fasting) were obtained on the third day after the last STZ treatment and every week thereafter to confirm high glucose level in STZ-treated mice.

2.2. Surgical procedure

Thirty days after STZ or vehicle injection, mice were anesthetized with Avertin (0.3 g/kg, ip; FVB: $n=20$; STZ: $n=16$). Supplemental doses of anesthetics (1/5 of the initial dose) were administered to prevent eye blink and withdrawal reflexes as needed. The femoral artery (left) and femoral vein (right) were exposed. Catheters (PE_{50}, with tips tapered to ~0.3 mm in diameter) were filled with heparinized saline and inserted into the femoral artery and vein. The catheters were then tunneled subcutaneously and exteriorized at the back of the neck (Dall'ago et al., 2007; Xue et al., 2005).

2.3. Baroreflex function

Approximately 24 h after catheterization surgery, the venous catheter was connected to a drug infusion line. Vasoactive agents phenylephrine (PE) or sodium nitroprusside (SNP) (Sigma, St. Louis, MO) were freshly prepared and diluted in 0.9% NaCl. Arterial blood pressure (ABP) was measured and recorded using a Powerlab Data Acquisition System (PowerLab/8 SP) that was connected to a pressure transducer (CB Sciences, BP100). HR was calculated from pulse pressure waves using the ratemeter function of the Chart 5.2 software. Mice were allowed to rest quietly for 60 min before being tested, during which time the baseline values of mean arterial blood pressure (MABP) and HR were recorded and averaged. To test baroreflex sensitivity, we performed two different assays.

First, microinfusions of PE or SNP were given through a micro-infusion pump (WPI SP101i). Various doses of PE or SNP (0.03, 0.05, 0.1, 0.2, 0.3, and 0.4 μg/min; volume 3–40 μl) were administered for 60 s in a random sequence. All doses of the same agent were given to each mouse on the same day but PE or SNP were given to different mice on different days. 30–40 min were allowed between doses, during which time MABP and HR returned to their original baseline levels. Shortly before the end of 60 s sustained microinfusion of PE or SNP at each dose, HR and MABP responses reached a plateau. The maximal HR response at the plateau relative to the baseline HR (ΔHR) in response to the MABP change relative to the baseline MABP (ΔMABP) was measured. For each of the vasoactive agents at each dose, HR and MABP responses were averaged over 5 s at the end of the 60 s sustained microinfusion of the agent (Lin et al., 2009). Then, the mean of the ΔHR and ΔMABP at each drug dose was calculated for each group. In some control and STZ mice, some premature heart beats were observed after large dose PE administration. Therefore, we restricted our data analysis to the periods during which premature heart beats were not found. Baroreflex sensitivity was assessed using the ratio of maximal ΔHR and ΔMABP (ΔHR/ΔMABP) at each drug dose. Baroreflex-mediated bradycardia was also assessed as ABP quickly increased when large doses of PE (0.3 μg/min) were administered, and baroreflex-mediated tachycardia was examined as ABP abruptly decreased when large doses of SNP (0.3 μg/min) were administered. Changes in HR (ΔHR) were measured and averaged over 0.5 s at every 5 mm Hg increase or decrease of MABP (ΔMABP = ±5 mm Hg). Then ΔHR was plotted as a function of ΔMABP to show the transient HR responses to changes in MABP.

2.4. Cardiac ganglia

Twenty-five days after STZ or vehicle treatment, mice (4–5 months of age, $n=6$/group) were injected with Fluoro-Gold (FG: i.p., 0.3 ml of 2 mg/ml per mouse) to label cardiac ganglia. Five days after FG injections, animals were anesthetized with sodium pentobarbital