Life Sciences 118 (2014) 263–267

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Late dual endothelin receptor blockade with bosentan restores impaired cerebrovascular function in diabetes

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ARTICLE INFO

Article history:
Received 22 October 2013
Accepted 31 December 2013
Available online 13 January 2014

Keywords:
Basilar artery
Middle cerebral artery
Myogenic tone
Endothelial function
Endothelin

ABSTRACT

Aims: Up-regulation of the endothelin (ET) system in type-2 diabetes increases contraction and decreases relaxation in basilar artery. We showed that 1) ET-receptor antagonism prevents diabetes-mediated cerebrovascular dysfunction; and 2) glycemic control prevents activation of the ET-system in diabetes. Here, our goal is to determine whether and to what extent glycemic control or ET-receptor antagonism reverses established cerebrovascular dysfunction in diabetes.

Main methods: Non-obese type-2 diabetic Goto–Kakizaki rats were administered either vehicle, metformin (300 mg/kg/day) or dual ET-receptor antagonist bosentan (100 mg/kg) for 4-weeks starting at 18-weeks after established cerebrovascular dysfunction (n = 5–6/group). Control group included vehicle-treated aged-matched Wistar rats. Blood glucose and pressure were monitored weekly. At termination, basilar arteries were collected and cumulative dose–response curves to ET-1 (0.1–500 nM), 5-HT (1–1000 nM) and acetylcholine (Ach, 0.1 nM–5 μM) were studied by wire myograph. Middle cerebral artery (MCA) myogenic reactivity and tone were measured using pressurized arteriograph.

Key findings: There was no difference in ET-1 and 5-HT-mediated constrictions. Endothelium-dependent relaxation was impaired in diabetes. Bosentan improved sensitivity to Ach as well as the maximum relaxation. Myogenic-tone is decreased over the course of the disease. Both treatments improved the ability of MCAs to develop tone at 80 mm Hg and only bosentan improved the tone at higher pressures.

Significance: These results suggest that contractile response is not affected by glycemic control or ET-receptor antagonism. Meanwhile, dual ET-receptor blockade is effective in partially improving endothelium-dependent relaxation and myogenic response in a blood pressure-independent manner even after established cerebrovascular dysfunction and offers therapeutic potential.

Introduction

Diabetes increases the risk and severity of cerebrovascular diseases such as ischemic stroke and vascular cognitive impairment (Kodl and Seaquist, 2008; Ergul et al., 2012). Diabetes is also associated with decreased cerebral blood flow, which is increasingly recognized as a major factor contributing to the development and progression of cognitive deficits in this population (Kodl and Seaquist, 2008). Undoubtedly, regulation of cerebrovascular tone is important for maintenance of proper blood flow (Faraci and Heistad, 1990). Myogenic and agonist-induced reactivity of the cerebral vasculature play a key role in controlling cerebral blood flow, and large arteries like basilar artery contribute significantly to regulation of cerebrovascular resistance (Faraci and Heistad, 1990). Thus, vascular dysfunction could contribute to cerebrovascular disease, and indeed, a number of studies have shown that diabetes alters myogenic tone and impairs endothelium-derived relaxation in experimental models (Dumont et al., 2003; Harris et al., 2008; Kelly-Cobbs et al., 2012). We have shown that myogenic tone is increased early in disease but as cerebral blood vessels remodel, they lose their ability to develop tone, and glycemic control started at the onset of diabetes prevents this change in myogenic tone (A. Kelly-Cobbs et al., 2011; A.I. Kelly-Cobbs et al., 2011). Whether and to what extent diabetes-mediated cerebrovascular dysfunction can be reversed if glycemic control is initiated later in the disease is not known.

It is well established that the potent vasoconstrictor endothelin-1 (ET-1) and cognate receptors, ET_A and ET_B (Goto et al., 1996), are activated in both clinical and experimental diabetes (Takahashi et al., 1990; Collier et al., 1992). ET_A receptors residing on the smooth muscle cell (SMC) produce vasoconstriction and mediate the proliferative effects of ET-1, while endothelial ET_B counteracts these effects. However,

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http://dx.doi.org/10.1016/j.lfs.2013.12.231

we have shown that 1) there is an upregulation of the VSMC ET_B receptors in the cerebrovasculature in diabetes (A. Kelly-Cobbs et al., 2011; A.I. Kelly-Cobbs et al., 2011); and 2) selective ET_A, selective ET_B or dual ET receptor blockade is vasculoprotective and prevents diabetes-mediated cerebrovascular dysfunction (Harris et al., 2005; A. Kelly-Cobbs et al., 2011; A.I. Kelly-Cobbs et al., 2011). We further demonstrated that glycemic control prevents activation of the ET system in diabetes (Sachidanandam et al., 2009), which is associated with improved vascular function (A. Kelly-Cobbs et al., 2011; A.I. Kelly-Cobbs et al., 2011). While these studies provided important evidence with regard to preventive cerebrovascular protective role of ET-1 antagonism, the therapeutic efficacy remained unknown. In this study, we tested the hypothesis that dual ET-1 receptor antagonism reverses established myogenic and endothelial dysfunction in diabetes.

Materials and methods

Animals

All experiments were performed using male Wistar rats (Harlan; Indianapolis, ID) and age-matched diabetic GK rats (In-house bred, derived from the Tampa colony or purchased from the Tampa colony, Taconic; Hudson, NY). We have chosen this non-obese model of type 2 diabetes because diabetes-induced cerebrovascular alterations can be studied in the absence of confounding comorbidities such as hypertension or hyperlipidemia. The animals were housed at the Georgia Regents University animal care facility that is approved by the American Association for Accreditation of Laboratory Animal Care. All protocols were approved by the institutional animal care and use committee. Animals were fed standard rat chow and tap water ad libitum.

Starting at 18 weeks of age after the development of diabetes-induced cerebrovascular dysfunction, the following groups (n = 5–6/group) were treated for 4 weeks: 1) GK vehicle, 2) GK + metformin (300 mg/kg/day in drinking water which we showed to achieve effective glycemic control in previous studies (Sachidanandam et al., 2009; Elgebaly et al., 2010)), 3) GK + bosentan (100 mg/kg/day by oral gavage), and 4) control Wistar vehicle. In additional groups (n = 5/group) of 10 and 18 week-old diabetic GK rats, myogenic reactivity was measured to investigate the impact of disease duration on tone development. Body weights and blood glucose measurements were taken biweekly. Blood glucose measurements were taken from tail vein samples using a commercially available glucometer (Freestyle, Abbott Diabetes Care, Inc.; Alameda, CA). Mean arterial pressure (MAP, mm Hg) was measured using the tail-cuff method. All animals were anesthetized with pentobarbital sodium (Fatal-Plus, Vortech Pharmaceuticals Ltd.; Dearborn, MI), exsanguinated via cardiac puncture, and decapitated to extract the brain. Middle cerebral arteries (MCA) and basilar arteries (BA) were isolated for functional studies with pressurized arteriograph and myograph, respectively.

Myogenic function studies

MCAs were quickly excised and used within 45 min of isolation to ensure viability of the vessels. A pressure arteriograph system (Living Systems; Burlington, VT) was used to evaluate MCA myogenic reactivity and tone. For these studies, MCA segments approximately 200–250 μm in diameter and proximal to the junction between the MCA and the inferior cerebral vein were used exclusively. Vessels were first mounted onto glass cannulas in an arteriograph chamber and HEPES bicarbonate buffer (in mM: 130 NaCl, 4 KCl, 1.2 $MgSO_4$, 4 $NaHCO_3$, 10 HEPES, 1.18 KH_2PO_4, 5.5 glucose, 1.8 $CaCl_2$) was circulated and maintained at 37 ± 0.5 °C. MCA segments were then pressurized at 60 mm Hg for 1 h to generate spontaneous tone. A video dimension analyzer connected to the arteriograph system was used to measure media thickness (MT) and lumen diameter (LD) at pressures ranging from 5 to 180 mm-Hg, in 20 mm-Hg increments. The first measurement was taken at 5 mm Hg because negative pressure is generated at 0 mm Hg, causing the vessel to collapse. All vessels were exposed to each pressure point for 5 min before readings were recorded. Pressure-diameter curves were obtained, first in the presence of Ca^{2+} to observe the vessels' contractile properties, and then in Ca^{2+}-free buffer with the addition of 10^{-7} M papaverine hydrochloride to evaluate the vessels' passive properties. Using the outer diameter (OD) measurements obtained in active conditions (in the presence of Ca^{2+}) and in passive conditions (in the absence of Ca^{2+}), Percent Myogenic Tone (% tone) = 1 − (Active OD / Passive OD) × 100 was calculated over the entire pressure range (20–180 mm-Hg) and tones at 80 and 120 mm-Hg were reported to represent mid and high end of the pressure curve, respectively.

Endothelial function studies

Isometric tension exerted by BAs was recorded via a force transducer using the wire-myograph technique (Danish Myo Technologies, Denmark). The myograph chambers were filled with Krebs buffer (NaCl 118.3, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.5 and Dextrose 11.1 mM), gassed with 95% O_2 and 5% CO_2 and maintained at 37 °C. Vessel segments were mounted in the chamber using 40 μm-thin wires and adjusted to a baseline tension of 0.4 g. Buffer was then switched to 70 mM KCl containing Krebs buffer in which NaCl concentration was reduced to achieve similar osmolarity and endothelial integrity was checked by relaxation response to 1 μM acetylcholine (Ach). Chambers were washed, refilled with regular Krebs buffer and cumulative dose–response curves to serotonin 5-HT (1–1000 nM) and ET-1 (0.1–500 nM) were generated. The force generated was expressed as % change of KCl. Endothelium-dependent relaxation to Ach (1 nM–1 μM) was assessed directly after ET-1 dose–response. Sensitivity (EC_{50}) and maximum response (R_{max}) values were calculated from the respective dose–response equations.

Statistical analysis

Results are given as mean ± SEM. For EC_{50} and R_{max} values, analysis of variance (ANOVA) was performed with a post-hoc Tukey test. A repeated measures ANOVA was used to determine group differences (Diabetic vs. Control) across the ET-1 or Ach concentrations. Post-hoc group comparisons at each concentration used a Tukey's adjustment for the multiple comparisons. Graphpad Prism 5.0 was used for all statistical tests performed.

Results

Effect of bosentan and metformin on metabolic profile

Body weight was slightly lower and blood glucose higher in diabetic animals as compared to control. Treatment with metformin did not affect body weight or blood pressure but bosentan increased blood pressure in diabetic animals (Table 1).

Effect of bosentan and metformin on contractile response

ET-1 mediated constriction is a dose dependent manner (Fig. 1A) but there was no difference among the groups with respect to maximal contraction or sensitivity to ET-1 (Fig. 1B and C). KCl and HT-induced contractions were also similar in all groups (data not shown).

Effect of bosentan and metformin on relaxation response

Endothelium-dependent relaxation to Ach was significantly impaired; i.e. maximum relaxation was less and EC_{50} was higher indicating reduced sensitivity, in diabetic animals as compared to age-matched control animals (Fig. 2A). While it did not reach statistical significance, only bosentan increased relaxation (Rmax) despite elevated blood pressure