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Functional changes in vascular reactivity to adenosine receptor activation in type I diabetic mice

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ABSTRACT

Activation of adenosine receptors has been implicated in several biological functions, including cardiovascular and renal function. Diabetes causes morphological and functional changes in the vasculature, resulting in abnormal responses to various stimuli. Recent studies have suggested that adenosine receptor expression and signaling are altered in disease states such as hypertension, diabetes. Using a streptozotocin (STZ) mouse model of type I diabetes (T1D), we investigated the functional changes in aorta and resistance mesenteric arteries to adenosine receptor agonist activation in T1D. Organ baths and DMT wire myographs were used for muscle tension measurements in isolated vascular rings, and western blotting was used for protein analysis. Concentration response curves to selective adenosine receptor agonists, including CCPA (A_1 receptor agonist), Cl-IBMECA (A_3 receptor agonist), CGS-21680 (A_{2A} receptor agonist), and BAY 60–6583 (A_{2B} receptor agonist), were performed. We found that diabetes did not affect adenosine receptor agonist-mediated relaxation or contraction in mesenteric arteries. However, aortas from diabetic mice exhibited a significant decrease ($P < 0.05$) in A_1 receptor-mediated vasoconstriction. In addition, the aortas from STZ-treated mice exhibited an increase in phenylephrine-mediated contraction (EC_{50} 7.40 ± 0.08 in STZ vs 6.89 ± 0.14 in vehicle; $P < 0.05$), while relaxation to A_{2A} receptor agonists (CGS-21680) tended to decrease in aortas from the STZ-treated group (not statistically significant). Our data suggest that changes in adenosine receptor(s) vascular reactivity in T1D is tissue specific, and the decrease in A_1 receptor-mediated aortic contraction could be a compensatory mechanism to counterbalance the increased adrenergic vascular contractility observed in aortas from diabetic mice.

1. Introduction

Adenosine is produced during conditions of metabolic stress and high cellular activity to increase oxygen supply and decrease oxygen consumption. Adenosine is mainly generated by the 5′-nucleotidases, which catalyze the dephosphorylation of adenosine monophosphate (AMP) into adenosine. Intracellular adenosine levels are mainly regulated by adenosine kinase, which converts adenosine into AMP, while extracellular adenosine levels are regulated by adenosine deaminase, which degrades adenosine to inosine (Blackburn, 2003; Ham and Evans, 2012; Wen and Xia, 2012). Adenosine binds to a family of four P1 G-protein coupled receptors; namely A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor. Their activation is implicated in the modulation of renal and cardiovascular function as well as erectile function (Headrick et al., 2013; Labazi et al., 2016b; Layland et al., 2014; Phatarpekar et al., 2010; Vallon and Osswald, 2009; Wen and Xia, 2012). Recently, adenosine was described as an endothelium-derived hyperpolarizing factor due to its ability to relax and hyperpolarize vascular smooth muscle cells (Ohta et al., 2013). Vascular studies from our laboratory and others have demonstrated that, while A_1 and A_3 adenosine receptor activation results in vasoconstriction, $A_{2A}$and A_{2B} adenosine receptor activation results in vasodilation (Ansari et al., 2007; El-Awady et al., 2011; El-Gowelli et al., 2013; Hein et al., 2013; Kunduri et al., 2013b; Labazi et al., 2016a, 2016b; Nayeem et al., 2008; Sanjani et al., 2011; Tawfik et al., 2005; Teng et al., 2013). However, the contribution of each receptor to overall vascular tone regulation can be animal- or tissue-specific. For instance, our laboratory and others demonstrated that adenosine-mediated vasorelaxation in murine mesenteric arteries is A_{2B} adenosine receptor-dependent (Teng et al., 2013; Wang et al., 2010), while others showed that adenosine-mediated vasorelaxation in

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rat and rabbit mesenteric arteries is mainly dependent upon the A_{2A} adenosine receptor (de Brito et al., 2002; Hiley et al., 1995).

Type I diabetes (T1D) is a metabolic disease characterized by a deficit in insulin secretion resulting in increased blood glucose levels. In the U.S., a recently published study showed that the prevalence of T1D in youth has increased by 21.1% (Hamman et al., 2014). Additionally, chronic diabetes causes damage to several organ systems. In the vasculature, the effect of diabetes can be divided into microvascular (retinopathy, nephropathy, and cardiomyopathy) and macrovascular (cardiovascular diseases and erectile dysfunction) complications, with cardiovascular disease being the leading cause of morbidity and mortality in diabetic patients (Shi and Vanhoutte, 2009). Few studies have reported that cell- and tissue-specific changes in adenosine receptor expression can be altered in disease states such as diabetes (Bender et al., 2009; Duarte et al., 2006; Grden et al., 2005, 2007; Labazi et al., 2016a; Pawelczyk et al., 2005). However, not much is known about the changes in adenosine receptor expression and/or signaling in the vasculature. In the present study, we sought to investigate the effect of T1D on adenosine receptor–mediated regulation of vascular tone in the aorta and mesenteric arteries.

2. Materials and methods

2.1. Drugs and solutions

Acetylcholine and phenylephrine, 1-[2-Chloro-6-[[(3-iodophenyl) methyl]amino]-9H-purin-9-yl]-1-deoxy-N-methyl-β-D-ribofuranuronamide(Cl-IBMECA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2-Chloro-N6-cyclopentyladenosine (CCPA) and 2-[[6-Amino-3,5-dicyano-4-[4-(cyclopropylmethoxy)phenyl]-2-pyridinyl] thio]-acetamide (BAY, Bay 60–6583) were purchased from Tocris Bioscience.

2.2. Animals

All experimental protocols were performed according to West Virginia University guidelines and with approval of the Animal Care and Use Committee.

2.3. Induction of diabetes

T1D was induced in 7 to 9 week-old C57BL/6 male mice following the protocol of the Animal Models of Diabetic Complications Consortium and using multiple low-dose streptozotocin (STZ; Sigma, St. Louis, MO) injections as previously described (Labazi et al., 2016a; Wu and Huan, 2008). Briefly, injections of 50 mg/kg body weight STZ dissolved in sodium citrate buffer (pH 4.5) were performed daily for 5 consecutive days after 4–5 h of fasting. Mice that served as vehicle controls were given the same volume per body weight of sodium citrate buffer. Mice with blood glucose levels > 250 mg/dl were considered diabetic. Twelve weeks post-STZ injections, animals were sacrificed for further experimentation (Oelze et al., 2011).

2.4. Functional studies in aortas and mesenteric arteries

Mice were euthanized with sodium pentobarbital (65 mg/kg i.p.) followed by thoracotomy. Thoracic aortas and the intestines were isolated from vehicle- and STZ-treated mice and placed in oxygenated Krebs-Henseleit buffer (pH 7.4) containing (in mM) 118 NaCl, 4.8 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 11 glucose, and 2.5 $CaCl_2$and maintained at 37 °C with continuous bubbling of 95% O_2-5% CO_2 (El-Awady et al., 2011; Kunduri et al., 2013a; Ponnoth et al., 2009).

Thoracic aortas were cleaned from fat and connective tissue then cut transversely into 2- to 3-mm rings. Aortic rings were mounted vertically between two stainless steel wire hooks and suspended in 6-ml organ baths containing Krebs-Henseleit buffer. For measurement of isometric tension, aortic rings were equilibrated for 60 min with a resting force of 1 g. Changes in tension were monitored continuously with a fixed range precision force transducer (TSD, 125 C; Biopac system) connected to a differential amplifier (DA 100B; Biopac system). The data were recorded using an MP100 Biopac digital acquisition system and analyzed using Acknowledge 3.5.7 software (Biopac system) (Ponnoth et al., 2009). After stabilization, rings were precontracted with 50 mM KCl to check the viability and contractility of individual aortic rings. Endothelial integrity was assessed by contraction with phenylephrine (PE, 10^{-6} M), followed by relaxation with acetylcholine (ACh, 10^{-6} M). In addition to concentration response curves (CRCs) to PE, CRCs to ACh and adenosine receptor agonists (CCPA, Bay 60–6583 (BAY), CGS21680 [CGS)] and Cl-IBMEC; 10^{-10} to 10^{-5} M were performed in PE-precontracted vessels.

First-order branches (~200 μm) of the superior mesentery arteries were isolated and cleaned of surrounding tissue. The arterial rings were mounted on an isometric myograph (Danish MyoTechology A/S, Aarhus, Denmark). Each vascular ring was stretched to a resting passive tension (200 mg) and allowed to equilibrate for at least 30 min. Viability of the vascular ring was verified by recording contraction after the addition of 50 mM KCl to the tissue bath. The integrity of the endothelium was confirmed by the addition of the endothelium-dependent vasodilator ACh (10^{-6} M) during the plateau phase of 10^{-6} M PE-induced contraction (Teng et al., 2013). CRCs to ACh and adenosine receptors agonists (CCPA, BAY,and Cl-IBMEC; 10^{-10} to 10^{-5} M) were performed in PE-precontracted vessels.

2.5. Protein expression

Western blot analyses were performed as described previously from this laboratory (Kunduri et al., 2013a). Aortas from vehicle and STZ-treated mice were homogenized with 150 μl radio-immuno precipitation assay buffer (Cell Signaling Technology; 20 mM Tris-HCl[pH 7.5],150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1 μg/ml leupeptin), vortexed, and centrifuged for 10 min at 13,800 G at 4 °C. Protein was measured using the Bradford dye procedure with bovine serum albumin as a standard (Bio-Rad Laboratories; Hercules, CA). Samples (25–30 μg of total protein) were loaded on slab gels (10% acrylamide; 1 mm thick), separated by SDS-PAGE, and transferred to nitrocellulose membranes (Hybond-ECL). Protein transfer was confirmed by visualization of prestained molecular weight markers (Bio-Rad). Membranes were blocked with 5% nonfat dry milk and incubated with A_1 adenosine receptor primary antibody (1:1000 dilution) (Sigma; St. Louis, MO); β-actin antibody (1:10,000 dilution) (Santa Cruz Biotechnology; Santa Cruz, CA) was used as an internal control to normalize the target protein expression in each lane. Membranes were developed using enhanced chemiluminescence (GE Healthcare) and x-ray film.

2.6. Statistical analysis

CRCs were analyzed using analysis of variance (ANOVA) followed by Bonferroni to compare between groups at the same concentration. In addition, an F-test was used for the estimation of EC_{50} values obtained from best-fit analysis using a nonlinear interactive fitting program for ACh and PE (GraphPad Prism, Graph Pad Software Inc.; San Diego, CA). Other parameters were analyzed using Student's *t*-test for significance. Data are expressed as means ± S.E.M. (n), where 'n' is the number of mice. Values of $P < 0.05$ were considered statistically significantly different.