Journal of Molecular and Cellular Cardiology 90 (2016) 30–37

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Enhanced A_{2A} adenosine receptor-mediated increase in coronary flow in type I diabetic mice

Hicham Labazi, Bunyen Teng, Zhichao Zhou, S. Jamal Mustafa *

Department of Physiology and Pharmacology, Center for Cardiovascular and Respiratory Sciences and Clinical Translational Science Institute, West Virginia University, Morgantown, WV, United States

ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form 9 November 2015
Accepted 30 November 2015
Available online 2 December 2015

Keywords:
Diabetes
coronary flow
A_{2A} adenosine receptor
NECA
CGS 21680

ABSTRACT

Adenosine A_{2A} receptor (A_{2A}AR) activation plays a major role in the regulation of coronary flow (CF). Recent studies from our laboratory and others have suggested that A_{2A}AR expression and/or signaling is altered in disease conditions. However, the coronary response to AR activation, in particular A_{2A}AR, in diabetes is not fully understood. In this study, we use an STZ mouse model of type 1 diabetes (T1D) to look at CF responses to the nonspecific AR agonist NECA and the A_{2A}AR specific agonist CGS 21680 *in-vivo* and *ex-vivo*. Using immunofluorescence, we also explored the effect of diabetes on A_{2A}AR expression in coronary arteries. NECA mediated increase in CF was significantly increased in hearts isolated from STZ-induced diabetic mice. In addition, both in *in-vivo* and *ex-vivo* responses to A_{2A}AR activation using CGS 21680 were significantly higher in diabetic mice when compared to their controls. Immunohistochemistry showed an upregulation of A_{2A}AR in both coronary smooth muscle and endothelial cells (~160% and ~140%, respectively). Our data suggest that diabetes resulted in an increased A_{2A}AR expression in coronary arteries which resulted in enhanced A_{2A}AR-mediated increase in CF observed in diabetic hearts. This is the first report implying that A_{2A}AR has a role in the regulation of CF in diabetes, supporting recent studies suggesting that the use of adenosine and its A_{2A} selective agonist (regadenoson, Lexiscan®) may not be appropriate for the detection of coronary artery diseases in T1D and the estimation of coronary reserve.

1. Introduction

Type 1 diabetes (T1D) is a metabolic disease characterized by an increase in blood glucose levels due to a deficit in insulin secretion. In this country, recent published study has shown that the prevalence of T1D in youth has increased by 21.1% [1]. Additionally, long-term diabetes causes damage to several organ systems. In the vasculature, diabetic complications can be divided into microvascular (retinopathy and nephropathy) and macrovascular (cardiovascular diseases and erectile dysfunction), with cardiovascular disease being the leading cause of morbidity and mortality in diabetic patients [2]. In the heart, vascular and endothelial dysfunction is a well-established complication of diabetes, resulting in coronary artery disease (CAD) and may be in part responsible for the increased incidence of ischemic heart disease in the diabetic population. One of the most important factors regulating coronary artery (CA) vascular tone is adenosine, which is a metabolite released locally under physiologic and pathophysiologic conditions. In diabetes, studies have shown a crucial role of adenosine signaling in the regulation of glucose homeostasis and pathophysiology of the disease. In a model of T1D, non-selective adenosine receptor ligand NECA was able to promote pancreatic β cell regeneration *in-vivo*, which was countered by gene ablation of A_{2A}AR, suggesting an important role for this receptor in promoting the restoration of normoglycemia [3–5]. Adenosine is an autocoid that exerts its effects through activation of four G-protein coupled receptors: A_1, A_{2A}, A_{2B}, and A_3. While activation of A_{2A}AR and A_{2B}AR results in vasodilation, A_1AR and A_3AR receptor activation results in vasoconstriction. Within the heart, adenosine plays an important role in cardiac contractility, coronary flow (CF), inflammation, cell growth, tissue remodeling, and substrate utilization [6]. In the coronary vasculature, vasodilation was shown to be predominantly mediated through A_{2A}AR activation, while A_1AR activation was shown to negatively regulate vasodilation in mice [6–12]. A_{2B}AR and A_3AR play a lesser role in the regulation of CF [6,11,13]. In the clinic, adenosine has long been used for super ventricular tachycardia as well as detection of CAD [14–16]. Because of adenosine's frequent undesirable side-effects, regadenoson (Lexiscan®, approved by FDA in 2008), an A_{2A}AR selective agonist, is preferred for myocardial perfusion imaging due to its minimal side effects [17]. Our laboratory has recently shown an

* Corresponding author at: Department of Physiology and Pharmacology, Morgantown, WV 26506-9104, United States
E-mail address: sjmustafa@hsc.wvu.edu (S.J. Mustafa).

http://dx.doi.org/10.1016/j.yjmcc.2015.11.033
0022-2828/

increase of A_{2A}AR-mediated CF in a mouse model of hyperlipidemia and/or atherosclerosis, suggesting that possible A_{2A}AR upregulation should be taken into consideration while using A_{2A}AR agonists for cardiac imaging to assess coronary reserve in disease states [18]. The effect of diabetes on AR subtype expression is not well studied, with only a few studies reporting cell and tissue specific changes in AR expression in the heart, kidney, and liver [19–21]. We and others have demonstrated that the A_{2A}AR plays a major role in the regulation of coronary microvascular dilation; however, no studies have investigated the effect of T1D on the AR regulation of CF. We sought to determine the effect of T1D on A_{2A}AR expression and on CF response to A_{2A} activation *in-vivo* and *ex-vivo* to examine the hypothesis that the A_{2A}AR-mediated increase in CF is impaired.

2. Methods

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

2.1. STZ mice and induction of diabetes

T1D was induced in 7 to 9-week-old male mice following the protocol of the Animal Models of Diabetic Complications Consortium using multiple low-dose streptozotocin (STZ; Sigma, St. Louis, MO) injections as previously described [22]. 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 to 5 h of fasting. Mice that served as vehicle controls were given the same volume per body weight of sodium citrate buffer. Blood glucose levels >300 mg/dL were considered diabetic. Eight weeks post-STZ injections, animals were killed for further experimentation [23].

2.2. Materials

5′-*N*-ethylcarboxamidoadenosine (NECA) and 4-[2-[[6-amino-9-(N-ethyl-β-D-ribofuranuronamidosyl)-9H-purin-2-yl]amino]ethyl]benzenepropanoic acid (CGS 21680) were purchased from Sigma-Aldrich (St. Louis, MO, USA). NECA and CGS-21680 were prepared as 10 mM stock solutions using DMSO (Sigma, St. Louis, MO) followed by serial dilutions with 50% DMSO and distilled water and a further dilution to the desired concentration was achieved with distilled water (final DMSO concentration of <1%) [9]. Anti-A_{2A}AR antibody was purchased from EMD Millipore. Alexa 533-conjugated goat anti-mouse secondary antibody was purchased from Invitrogen, and DRAQ5 was purchased from Abcam.

2.3. Langendorff-perfused mouse heart preparation

Isolated heart experiments were performed in accordance with our previously published methods [9,11–13]. In brief, mice were anesthetized with pentobarbital sodium (50 mg/kg ip). A thoracotomy was performed, and hearts were removed into heparinized (5 U/mL), ice-cold Krebs-Hensleit (KH) buffer. Hearts were rapidly perfused retrogradely through the aorta cannulated with a 20-gauge, blunt-ended needle at a constant pressure of 80 mm Hg and continuously gassed with 95% O_2–5% CO_2 KH buffer containing 119 mM NaCl, 11 mM glucose, 22 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 2 mM pyruvate, and 0.5 mM EDTA at 37 °C in a standard Langendorff fashion and allowed to beat spontaneously. The left atrium was removed, and the left ventricle (LV) was vented with a small polyethylene apical drain. A water-filled balloon made of plastic wrap was inserted into the LV across the mitral valve, which was connected to a fluid-filled pressure transducer by polyethylene tubing for the continuous measurement of LV developed pressure (LVDP). CF was measured *via* a Transonic flow probe (Transonic Systems, Ithaca, NY) in the aortic perfusion line. Baseline coronary flow, LVDP, and heart rate (HR; derived from the ventricular pressure trace) were monitored for 30–40 min during the equilibration period and recorded on a Power Lab data-acquisition system (AD Instruments, Colorado Springs, CO) [9–13,18,24].

2.4. Langendorff experimental protocols

After equilibrium, concentration response curves (CRC) (10^{-10}–10^{-6} M) to non-selective AR agonist NECA and to the A_{2A}AR agonist CGS 21680 were acquired in perfused hearts from wild type mice. These agonists were infused into the coronary perfusate through an injection port directly proximal to the aortic cannula. The infusion rate was controlled to a maximum of 1% of the total CF by a microinjection infusion pump. Each concentration of either agonist was infused for 5 min or until the flow is plateaued followed by a minimum of 5 min of perfusion for drug washout [12,13,18,24].

2.5. In-vivo assessment of echocardiography and coronary blood flow Doppler measurement

Each mouse was anesthetized in an induction chamber with inhalant isoflurane at 3% in 100% oxygen. When fully anesthetized, the mouse was transferred to dorsal recumbency, placed on a heated imaging platform, and maintained at 1–1.25% isoflurane for the duration of the experiment. The hair of the mouse chest wall was carefully removed, and warm electrode gel was applied to the limb leads, allowing for an electrocardiogram and the respiration rate to be recorded during ultrasound imaging. A rectal probe was used to monitor the temperature of the mouse. Ultrasound images were acquired using MS550D transducer (22–55 MHz) on the Vevo2100 Imaging System (Visual Sonics, Toronto, Canada). Placing the transducer to the left of the sternum allowed us to obtain images of the aortic outflow tract, the apex of the heart, and LV along its longest axis (i.e., long-axis B-mode images). Once all long-axis B-mode images were attained, the transducer was rotated 90 degrees to acquire short-axis B-mode images at the mid-papillary muscle level then moved up till left coronary artery (LCA) was visible for the measurement of the size of the vessel. Then transducer was rotated back to long axis parasternal view with the probe lateralized and the ultrasound beam anteriorly tilted. In this image window, the entire LCA, from the aortic sinus to the distal branch site, could be visualized using color Doppler echocardiography. The course of the LCA was typically parallel to the Doppler beam, which also facilitates Doppler measurements without any angle correction. Then the system was switched to pulse-wave Doppler mode with a gate size of 0.65 mm. CF signals were identified on the Doppler spectral display by flow toward the probe peaking in early diastole and then decaying and being minimal during systole as illustrated in Fig. 3. The flow velocity measurements were made at the same vessel site at baseline and during CGS 21680-induced hyperemia (Fig. 4). Measurements were averaged from three cardiac cycles.

Coronary Blood Flow (CBF) was calculated using following formula:

$$\text{Flow}_{\text{CBF}}(\text{mL/ min}) = \left((\pi/4) \times D^2 \times \text{VTI} \times \text{HR}\right)/1000$$

where D is the internal coronary diameter (in mm) measured in B-mode ultrasound images, VTI is the velocity–time–integral (in mm), or area under the curve of the Doppler blood flow velocity tracing, and HR is heart rate. Coronary Flow Reserve (CFR) = $\text{CBF}_{\text{CGS 21680}}/\text{CBF}_{\text{baseline}}$ where $\text{CBF}_{\text{CGS 21680}}$ is the peak coronary flow measured after 0.5 mg/kg CGS 21680 femoral vein IV injection. The peak CBF was usually reached within 1 ½ mins after injection. After peak CBF was reached, cardiac function and coronary artery size were measured again for the measurement of the CGS 21680 effect [25].