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

Hicham Labazi[a], **Bunyen Teng**[a], and **S. Jamal Mustafa**[a,b,*]

[a]Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV, USA

[b]WV Center for Tranlational Science Institute, Morgantown, WV, USA

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.

*Corresponding author: S. Jamal Mustafa, PhD, Department of Physiology, Pharmacology and Neuroanatomy, WVU School of Medicine, PO Box 9229, sjmustafa@hsc.wvu.edu.

Current address for Dr. Hicham Labazi: Department of Physiology, University of Arizona, 1501 N. Campbell Ave., Tucson, AZ 85724, hlabazi@yahoo.fr

Current address for Dr. BunyenTeng: Coagulation and Blood Research Task Area, U.S. Army Institute of Surgical Research, 3698 Chambers Pass, BLDG 3610, JBSA-Fort Sam Houston, TX 78234-7767, bunyen.teng.ctr@mail.mil

Authors Contributions:

HL conceived and designed research; HL and BT performed experiments and analyzed data; HL drafted the manuscript; HL, BT and SJM revised and edited the manuscript

Conflicts of interest

The authors declare no conflicts of interest.

Keywords

Adenosine; adenosine receptors; diabetes; vascular function

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; Labazi et al., 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 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 characterizedby 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 to 5h 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 mMKCl 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 concentrationresponse 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 mins. 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.

3. Results

3.1. Effect of diabetes on body weight and blood glucose

Compared to age-matched control mice, diabetic mice exhibited a significant decrease in body weight (27.86 g ± 0.53 in vehicle vs 23.25g ± 0.29 in STZ; P<0.05). As expected, STZ injections resulted in significant increases in blood glucose levels compared to vehicle-injected mice (136.7 mg/dl ± 4.2 in vehicle vs 489.4 mg/dl ± 13.1 in STZ; P<0.05).

3.2 Effect of diabetes on contraction and relaxation

There was no significant difference in relaxation to ACh (70.6 % ± 2.9 in vehicle vs 65.6 ± 2 in STZ at 10^{-6} M; P=0.16) and contraction to50 mM KCl (0.50 g± 0.04 in vehicle vs 0.56 ± 0.002 in STZ; P=0.12). However, aortas isolated from diabetic mice exhibited a significant increase in contractility to the α1-adrenergic receptor agonist PE (91.4% ± 1.9 in vehicle vs 105.9% ± 3.5 in STZ at 10^{-6} M; P<0.05) (Fig. 1).

3.3. Aortas from diabetic mice exhibited decreased contraction to A_1 adenosine receptor activation

A_1 adenosine receptor activation resulted in a dose-dependent increase in aortic contraction; however, aortas from STZ-treated mice exhibited a significant decrease in A_1 adenosine receptor-mediated contraction to CCPA when compared to vehicle (Fig. 2). Conversely, A_3 adenosine receptor contraction to its agonist, Cl-IBEMCA, was not different between aortic rings isolated from vehicle or diabetic mice (Fig. 2). Although aortas isolated from vehicle-treated mice tended to have an increased relaxation to A_{2A} adenosine receptor agonist CGS at high concentrations (10^{-6} and 10^{-5} M), it was not significantly different between the aortas isolated from vehicle-and STZ-treated mice (P=0.19 and P=0.17, respectively; Fig. 2). In addition, relaxation to the A_{2B} adenosine receptor agonist BAY was not different between the two groups (Fig. 2).

3.4. Aortas from diabetic mice exhibited increased contraction to α1-adrenergic receptor activation

While we did not see a significant difference in endothelium-mediated vasorelaxation to ACh between aortas from vehicle- and STZ-treated mice (EC50: 7.06 ± 0.09 in vehicle vs 6.88 ± 0.09 inSTZ; P>0.05; Fig. 3), the aortas from STZ-treated mice exhibited a significant increase in contraction to the α1-adrenergic receptor agonist PE (EC_{50}: 6.89 ± 0.14 in vehicle vs 7.40 ± 0.08 in STZ; P<0.05; Fig. 3).

3.5. Mesenteric artery responses to adenosine receptor activation were not affected by diabetes

The mesenteric vascular response to adenosine receptor activation with the adenosine receptor agonists CCPA (A_1 adenosine receptor), Cl-IBEMCA(A_3 adenosine receptor), and BAY (A_{2B} adenosine receptor) were not different between vehicle- and STZ-treated mice (Fig. 4). Previous studies from our laboratory have shown that A_{2A} adenosine receptor activation does not affect vascular tone in mesenteric arteries (Teng et al., 2013).

Mesenteric artery contraction responses to PE were not different between the two groups (251.3% ± 34.9 KCl contraction in vehicle vs 265.4% ± 53.9 KCl contraction in STZ; n=10; P>0.05, data not shown). To investigate whether diabetes affects endothelium-mediated vasorelaxation in diabetic mesenteric arteries, CRC to ACh was performed. ACh-mediated vasorelaxation in mesenteric arteries isolated from diabetic mice was not different from that of control mice (EC_{50}: 7.34 ± 0.17 in vehicle vs 7.62 ± 0.21 in STZ, data not shown).

3.6. Aortic A_1 adenosine receptor protein expression was not affected by diabetes

Since diabetes affects aortic responses to A_1 adenosine receptors, we looked at the expression of A_1 adenosine receptor. A_1 adenosine receptor expression in aortas isolated from diabetic mice was not significantly different from that of the vehicle-treated mice (Fig. 5).

4. Discussion

In the present study, we aimed to elucidate the contribution of adenosine receptors to vascular tone in mice with T1D. We showed that diabetes resulted in decreased A_1 adenosine receptor-mediated contraction in the aorta, which was accompanied by increased aortic contraction to PE. However, A_1 adenosine receptor-mediated responses in mesenteric arteries were not different between vehicle- and STZ-treated mice, suggesting a differential vascular bed effect of diabetes on adenosine receptor function. Interestingly, A_1 adenosine receptor expression in the aorta was not different between the two groups, suggesting a possibly decreased sensitivity of A_1 adenosine receptor to its agonist in the aortas of diabetic mice. Our results are in concordance with earlier studies showing that, in STZ-treated diabetic rats, there was a decrease in A_1 adenosine receptor sensitivity to adenosine and impaired Gi coupling, with no change in A_1 adenosine receptor expression in adipocytes (Barrington et al., 1996; Green and Johnson, 1991). Furthermore, T1D had a tissue-specific effect on A_1 adenosine receptor sensitivity (Barrington et al., 1996), which may be the case in the present study as we did not see a difference in response to A_1 adenosine receptor activation in mesenteric arteries, contrary to aortas. The disconnect between the expression and activity/signaling of adenosine receptors is not novel. In fact, our laboratory has previously shown that, while mesenteric arteries express A_{2A} adenosine receptor, this adenosine receptor subtype does not contribute to the regulation of their vascular tone (Teng et al., 2013). Also, another study showed that, while the coronary arteries express A_{2B} adenosine receptor, this receptor did not play an active role in the endogenous regulation of coronary blood flow (Berwick et al., 2010). Interestingly, the same group demonstrated that obesity and metabolic syndrome augmented the contribution of A_{2B} adenosine receptorto adenosine-induced dilation, which was surprisingly associated with a decrease in coronary A_{2B} adenosine receptor protein expression (Bender et al., 2009). Together, these data, with other studies, suggest that diseases such as diabetes may alter receptor sensitivity and/or signaling independent of receptor expression levels. Unfortunately, little is known about the posttranslational modifications of adenosine receptors during both healthy and disease conditions, as is how these modifications (such asO-linked N-acetylglucosamineprotein modification in diabetes) may affect the receptor’s intracellular signaling pathways and activity (Palmer and Stiles, 1999).

As expected, STZ treatment resulted in an increase in blood glucose levels, accompanied by a significant decrease in body weight. These effects have been well documented by our laboratory and many others (Grden et al., 2005; 2007; Labazi et al., 2016a; Pawelczyk et al., 2005). While we did not observe differences in endothelium-mediated vasorelaxation in aortas and mesenteric arteries from vehicle- and STZ-treated groups, contractile responses to the adrenergic receptor agonist PE was significantly higher in aortas from diabetic mice. These data are in concordance with previous studies (Rehman et al., 2014; Xie et al., 2010). The observed decreased contraction to the A_1 adenosine receptor agonist may also be a compensatory mechanism to counteract increased aortic contractility to other agonists, such as PE, in diabetes to regulate vascular tone, at least at this early stage of T1D.

The newly considered notion that adenosine levels and adenosine receptor expression are dramatically changed in pathological conditions allowed for a new proposed role for the adenosine receptors. However, our understanding of the contribution of adenosine receptors to the physiology and pathophysiology of the cardiovascular system is still at an early stage, and the findings in the field seemed contradictory. For instance, increased adenosine levels and activation of A_{2B} adenosine receptor signaling was shown to be protective during diabetic nephropathy (Tak et al., 2014). However, increased adenosine and expression of A_{2B} adenosine receptor was shown to contribute to hypertension in chronic kidney disease (Zhang et al., 2013). This contradictory role of elevated adenosine levels and A_{2B} adenosine receptor signaling can be explained by the fact that A_{2B} adenosine receptor signaling can be tissue specific (endothelial cells vs whole kidney) or disease specific (diabetes vs hypertension). In addition, adenosine receptor signaling pathways are still under investigation.

In the vasculature, it is known that A_{2A} and A_{2B} adenosine receptors are stimulatory for adenylyl-cyclase, leading to cyclic-AMP (cAMP) production and vasodilation, while the A_1 and A_3 adenosine receptors are linked to the inhibitory G_i, suppressing adenylyl-cyclase and favoring contraction. However, studies have shown that pathways associated with increased vascular contraction, such as PKC and MAPK, can also be activated by A_{2A} and A_{2B} adenosine receptors. This was shown to be independent of Gs and was dependent upon Gi or Gq activation(Cohen et al., 2010; Schulte and Fredholm, 2003). The ability of theses receptors to potentially trigger multiple signaling pathways is intriguing and raises the question of if these signaling pathways are tissue specific and whether disease states, such as hypertension or diabetes, can favor one pathway over another. This may also explain the contradictory role of these receptors in attenuating versus exacerbating the disease (Tak et al., 2014; Zhang et al., 2013). Thus, it is imperative to study adenosine signaling in disease states to understand their contribution to the amelioration or exacerbation of the disease.

In an STZ-treated animal model, A_1 adenosine receptor expression was increased in the kidney (Pawelczyk et al., 2005), while its expression in pancreatic α-cells was diminished in a non obese diabetic mouse model (Yip et al., 2013), suggesting that its expression may be affected in diabetes. In the present study, we did not see a difference in A_1 adenosine receptor expression in the aorta, although the vascular response to A_1 adenosine receptor agonist was attenuated in aortic rings from diabetic mice. This may be a result of altered A_1 adenosine receptor signaling through decreased A_1 adenosine receptor sensitivity to its

agonist or its coupling to Gi, which was shown to be impaired in T1D (Barrington et al., 1996; Green and Johnson, 1991). In addition, T1D was shown to be associated with a decreased expression of Gi in rat hepatocytes, which may also result in decreased A_1 adenosine receptor signaling (Gawler et al., 1987). In addition, posttranslational modifications that are associated with diabetes, such as O-Glc-N-acylation, may also affect receptor signaling.

5. Conclusion

Other studies have shown that, despite apparent vascular dysfunction, blood pressure in STZ-treated animals is not significantly different from that of control animals (Nacci et al., 2009; Tojo et al., 2016). In this study, we showed that vascular A_1 adenosine receptor signaling was reduced in diabetes, and this may be due to a compensatory mechanism to counteract increased vascular contractility observed in diabetes and prevent increases in blood pressure (see Fig. 6). Our present study also showed a disconnect between receptor expression and signaling, which has also been previously shown by others(Bender et al., 2009). Thus, future studies investigating post-translational modifications and/or downstream receptor signaling pathways are crucial to understanding the potential role of the A_1 adenosine receptor and may explain the discrepancy observed between adenosine receptor expression and signaling in cardiovascular physiology and pathophysiology.

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References

Ansari HR, Nadeem A, Tilley SL, Mustafa SJ. Involvement of COX-1 in A(3) adenosine receptor-mediated contraction through endothelium in mice aorta. Am J Physiol-Heart C. 2007; 293:H3448–H3455.

Barrington WW, Crum M, Forst C, Scheetz M, Weide LG. Desensitization of the adipocyte A(1) adenosine receptor during untreated experimental diabetes mellitus. Endocrine. 1996; 4:199–205. [PubMed: 21153275]

Bender SB, Tune JD, Borbouse L, Long X, Sturek M, Laughlin MH. Altered mechanism of adenosine-induced coronary arteriolar dilation in early-stage metabolic syndrome. Exp Biol Med (Maywood). 2009; 234:683–692. [PubMed: 19307464]

Berwick ZC, Payne GA, Lynch B, Dick GM, Sturek M, Tune JD. Contribution of adenosine A(2A) and A(2B) receptors to ischemic coronary dilation: role of K(V) and K(ATP) channels. Microcirculation. 2010; 17:600–607. [PubMed: 21044214]

Blackburn MR. Too much of a good thing: adenosine overload in adenosine-deaminase-deficient mice. Trends Pharmacol Sci. 2003; 24:66–70. [PubMed: 12559769]

Cohen MV, Yang X, Downey JM. A(2b) adenosine receptors can change their spots. Br J Pharmacol. 2010; 159:1595–1597. [PubMed: 20388188]

de Brito MT, Canto A, Correia JH, Cunha RA, Marques MC. Adenosine A(2A) receptors in portal hypertension: their role in the abnormal response to adenosine of the cranial mesenteric artery in rabbits. Br J Pharmacol. 2002; 135:1324–1330. [PubMed: 11877342]

Duarte JM, Oliveira CR, Ambrosio AF, Cunha RA. Modification of adenosine A1 and A2A receptor density in the hippocampus of streptozotocin-induced diabetic rats. Neurochem Int. 2006; 48:144–150. [PubMed: 16256246]

El-Awady MS, Ansari HR, Fil D, Tilley SL, Mustafa SJ. NADPH Oxidase Pathway Is Involved in Aortic Contraction Induced by A(3) Adenosine Receptor in Mice. Journal of Pharmacology and Experimental Therapeutics. 2011; 338:711–717. [PubMed: 21606175]

El-Gowelli HM, El-Gowilly SM, Elsalakawy LK, El-Mas MM. Nitric oxide synthase/K+ channel cascade triggers the adenosine A(2B) receptor-sensitive renal vasodilation in female rats. Eur J Pharmacol. 2013; 702:116–125. [PubMed: 23396225]

Gawler D, Milligan G, Spiegel AM, Unson CG, Houslay MD. Abolition of the expression of inhibitory guanine nucleotide regulatory protein Gi activity in diabetes. Nature. 1987; 327:229–232. [PubMed: 3106832]

Grden M, Podgorska M, Szutowicz A, Pawelczyk T. Altered expression of adenosine receptors in heart of diabetic rat. J Physiol Pharmacol. 2005; 56:587–597. [PubMed: 16391416]

Grden M, Podgorska M, Szutowicz A, Pawelczyk T. Diabetes-induced alterations of adenosine receptors expression level in rat liver. Exp Mol Pathol. 2007; 83:392–398. [PubMed: 17490639]

Green A, Johnson JL. Evidence for impaired coupling of receptors to Gi protein in adipocytes from streptozocin-induced diabetic rats. Diabetes. 1991; 40:88–94. [PubMed: 1849851]

Ham J, Evans BA. An emerging role for adenosine and its receptors in bone homeostasis. Front Endocrinol (Lausanne). 2012; 3:113. eCollection. [PubMed: 23024635]

Hamman RF, Bell RA, Dabelea D, D'Agostino RB Jr, Dolan L, Imperatore G, Lawrence JM, Linder B, Marcovina SM, Mayer-Davis EJ, Pihoker C, Rodriguez BL, Saydah S. The SEARCH for Diabetes in Youth study: rationale, findings, and future directions. Diabetes Care. 2014; 37:3336–3344. [PubMed: 25414389]

Headrick JP, Ashton KJ, Rose'meyer RB, Peart JN. Cardiovascular adenosine receptors: expression, actions and interactions. Pharmacol Ther. 2013; 140:92–111. [PubMed: 23764371]

Hein TW, Xu W, Ren Y, Kuo L. Cellular signalling pathways mediating dilation of porcine pial arterioles to adenosine A(2)A receptor activation. Cardiovasc Res. 2013; 99:156–163. [PubMed: 23539502]

Hiley CR, Bottrill FE, Warnock J, Richardson PJ. Effects of pH on responses to adenosine, CGS 21680, carbachol and nitroprusside in the isolated perfused superior mesenteric arterial bed of the rat. Br J Pharmacol. 1995; 116:2641–2646. [PubMed: 8590983]

Kunduri SS, Mustafa SJ, Ponnoth DS, Dick GM, Nayeem MA. Adenosine A1 receptors link to smooth muscle contraction via CYP4a, protein kinase C-alpha, and ERK1/2. J Cardiovasc Pharmacol. 2013a; 62:78–83. [PubMed: 23519140]

Kunduri SS, Mustafa SJ, Ponnoth DS, Dick GM, Nayeem MA. Adenosine A(1) Receptors Link to Smooth Muscle Contraction Via CYP4a, protein kinase C-, and ERK1/2. J Cardiovasc Pharm. 2013b; 62:78–83.

Labazi H, Teng B, Zhou Z, Mustafa SJ. Enhanced A2A adenosine receptor-mediated increase in coronary flow in type I diabetic mice. J Mol Cell Cardiol. 2016a; 90:30–37. [PubMed: 26654777]

Labazi H, Tilley SL, Ledent C, Mustafa SJ. Role of Adenosine Receptor(s) in the Control of Vascular Tone in the Mouse Pudendal Artery. Journal of Pharmacology and Experimental Therapeutics. 2016b; 356:673–680. [PubMed: 26718241]

Layland J, Carrick D, Lee M, Oldroyd K, Berry C. Adenosine: physiology, pharmacology, and clinical applications. JACC Cardiovasc Interv. 2014; 7:581–591. [PubMed: 24835328]

Nacci C, Tarquinio M, De Benedictis L, Mauro A, Zigrino A, Carratu MR, Quon MJ, Montagnani M. Endothelial dysfunction in mice with streptozotocin-induced type 1 diabetes is opposed by compensatory overexpression of cyclooxygenase-2 in the vasculature. Endocrinology. 2009; 150:849–861. [PubMed: 18845644]

Nayeem MA, Poloyac SM, Falck JR, Zeldin DC, Ledent C, Ponnoth DS, Ansari HR, Mustafa SJ. Role of CYP epoxygenases in A(2A)AR-mediated relaxation using A(2A)AR-null and wild-type mice. Am J Physiol-Heart C. 2008; 295:H2068–H2078.

Oelze M, Knorr M, Schuhmacher S, Heeren T, Otto C, Schulz E, Reifenberg K, Wenzel P, Munzel T, Daiber A. Vascular dysfunction in streptozotocin-induced experimental diabetes strictly depends on insulin deficiency. J Vasc Res. 2011; 48:275–284. [PubMed: 21273782]

Ohta M, Toyama K, Gutterman DD, Campbell WB, Lemaitre V, Teraoka R, Miura H. Ecto-5′-Nucleotidase, CD73, Is an Endothelium-Derived Hyperpolarizing Factor Synthase. Arterioscl Throm Vas. 2013; 33:629–636.

Palmer TM, Stiles GL. Stimulation of A(2A) adenosine receptor phosphorylation by protein kinase C activation: evidence for regulation by multiple protein kinase C isoforms. Biochemistry. 1999; 38:14833–14842. [PubMed: 10555965]

Pawelczyk T, Grden M, Rzepko R, Sakowicz M, Szutowicz A. Region-specific alterations of adenosine receptors expression level in kidney of diabetic rat. Am J Pathol. 2005; 167:315–325. [PubMed: 16049319]

Phatarpekar PV, Wen J, Xia Y. Role of adenosine signaling in penile erection and erectile disorders. Journal of Sexual Medicine. 2010; 7:3553–3564. [PubMed: 19889148]

Ponnoth DS, Sanjani MS, Ledent C, Roush K, Krahn T, Mustafa SJ. Absence of adenosine-mediated aortic relaxation in A(2A) adenosine receptor knockout mice. Am J Physiol Heart Circ Physiol. 2009; 297:H1655–1660. [PubMed: 19749167]

Rehman AU, Dugic E, Benham C, Lione L, Mackenzie LS. Selective inhibition of NADPH oxidase reverses the over contraction of diabetic rat aorta. Redox Biol. 2014; 2:61–64. [PubMed: 25460721]

Sanjani MS, Teng BY, Krahn T, Tilley S, Ledent C, Mustafa SJ. Contributions of A(2A) and A(2B) adenosine receptors in coronary flow responses in relation to the K-ATP channel using A(2B) and A(2A/2B) double-knockout mice. Am J Physiol-Heart C. 2011; 301:H2322–H2333.

Schulte G, Fredholm BB. Signalling from adenosine receptors to mitogen-activated protein kinases. Cell Signal. 2003; 15:813–827. [PubMed: 12834807]

Shi Y, Vanhoutte PM. Reactive oxygen-derived free radicals are key to the endothelial dysfunction of diabetes. J Diabetes. 2009; 1:151–162. [PubMed: 20923534]

Tak E, Ridyard D, Kim JH, Zimmerman M, Werner T, Wang XX, Shabeka U, Seo SW, Christians U, Klawitter J, Moldovan R, Garcia G, Levi M, Haase V, Ravid K, Eltzschig HK, Grenz A. CD73-dependent generation of adenosine and endothelial Adora2b signaling attenuate diabetic nephropathy. J Am Soc Nephrol. 2014; 25:547–563. [PubMed: 24262796]

Tawfik HE, Schnermann J, Oldenburg PJ, Mustafa SJ. Role of A1 adenosine receptors in regulation of vascular tone. Am J Physiol Heart Circ Physiol. 2005; 288:H1411–1416. [PubMed: 15539423]

Teng B, Fil D, Tilley SL, Ledent C, Krahn T, Mustafa SJ. Functional and RNA expression profile of adenosine receptor subtypes in mouse mesenteric arteries. J Cardiovasc Pharmacol. 2013; 61:70–76. [PubMed: 23288107]

Tojo A, Kinugasa S, Fujita T, Wilcox CS. A local renal renin-angiotensin system activation via renal uptake of prorenin and angiotensinogen in diabetic rats. Diabetes Metab Syndr Obes. 2016; 9:1–10. [PubMed: 26848273]

Vallon V, Osswald H. Adenosine receptors and the kidney. Handb Exp Pharmacol. 2009:443–470. [PubMed: 19639291]

Wang Y, Yang JN, Arner A, Boels PJ, Fredholm BB. Adenosine A(1) receptors and vascular reactivity. Acta Physiol (Oxf). 2010; 199:211–220. [PubMed: 20132147]

Wen J, Xia Y. Adenosine signaling: good or bad in erectile function? Arterioscler Thromb Vasc Biol. 2012; 32:845–850. [PubMed: 22423035]

Wu KK, Huan Y. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol. 2008; Chapter 5(Unit 5):47.

Xie Z, Gong MC, Su W, Xie D, Turk J, Guo Z. Role of calcium-independent phospholipase A2beta in high glucose-induced activation of RhoA, Rho kinase, and CPI-17 in cultured vascular smooth muscle cells and vascular smooth muscle hypercontractility in diabetic animals. J Biol Chem. 2010; 285:8628–8638. [PubMed: 20086008]

Yip L, Taylor C, Whiting CC, Fathman CG. Diminished adenosine A1 receptor expression in pancreatic alpha-cells may contribute to the pathology of type 1 diabetes. Diabetes. 2013; 62:4208–4219. [PubMed: 24264405]

Zhang W, Zhang Y, Wang W, Dai Y, Ning C, Luo R, Sun K, Glover L, Grenz A, Sun H, Tao L, Zhang W, Colgan SP, Blackburn MR, Eltzschig HK, Kellems RE, Xia Y. Elevated ecto-5′-nucleotidase-mediated increased renal adenosine signaling via A2B adenosine receptor contributes to chronic hypertension. Circ Res. 2013; 112:1466–1478. [PubMed: 23584256]

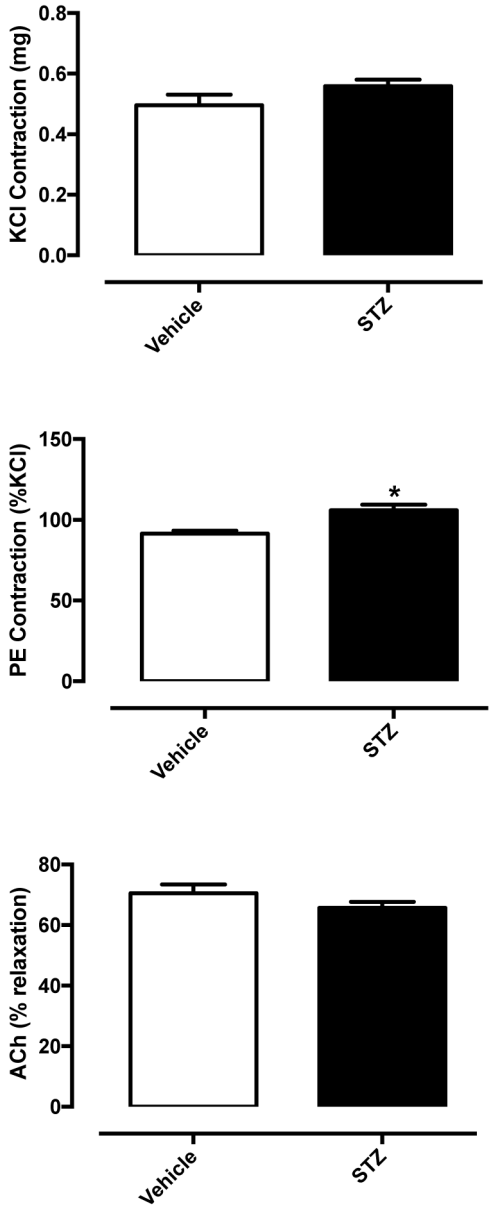

Fig. 1.

Aortas from diabetic mice exhibited an increased contraction to the α1-adrenergic receptor agonist PE (middle panel). No difference between the two groups was observed in contraction to 50 mM KCl (top panel) or relaxation to 10^{-6} M ACh (bottom panel) in PE pre-contracted aortic rings. Data are represented as mean ± S.E.M. (n=13–19). *P<0.05 vs. vehicle.

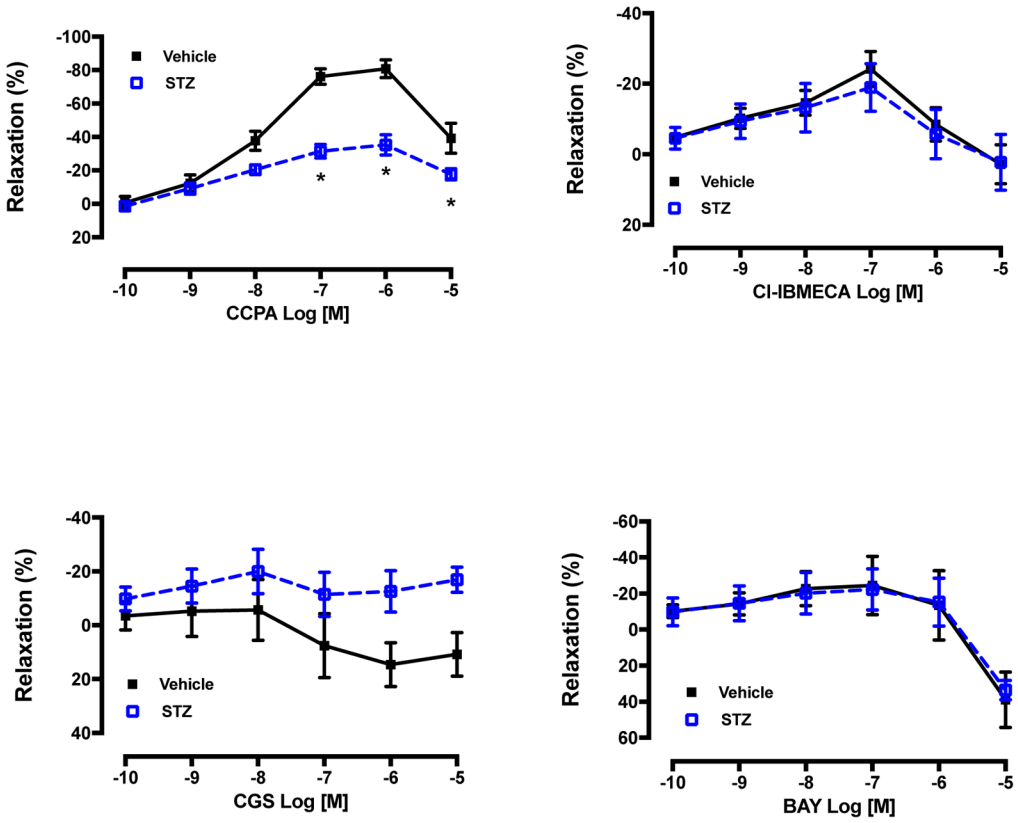

Fig. 2.

Aortas isolated from diabetic mice exhibited decreased contraction to the A_1 adenosine receptor agonist CCPA (top left panel). CRCs to CCPA, Cl-IBMECA, BAY 60-6583. and CGS 21680 were performed in aortas isolated from vehicle- and STZ-treated mice. Data are represented as mean ± S.E.M. (n=5–9). *P< 0.05vs vehicle.

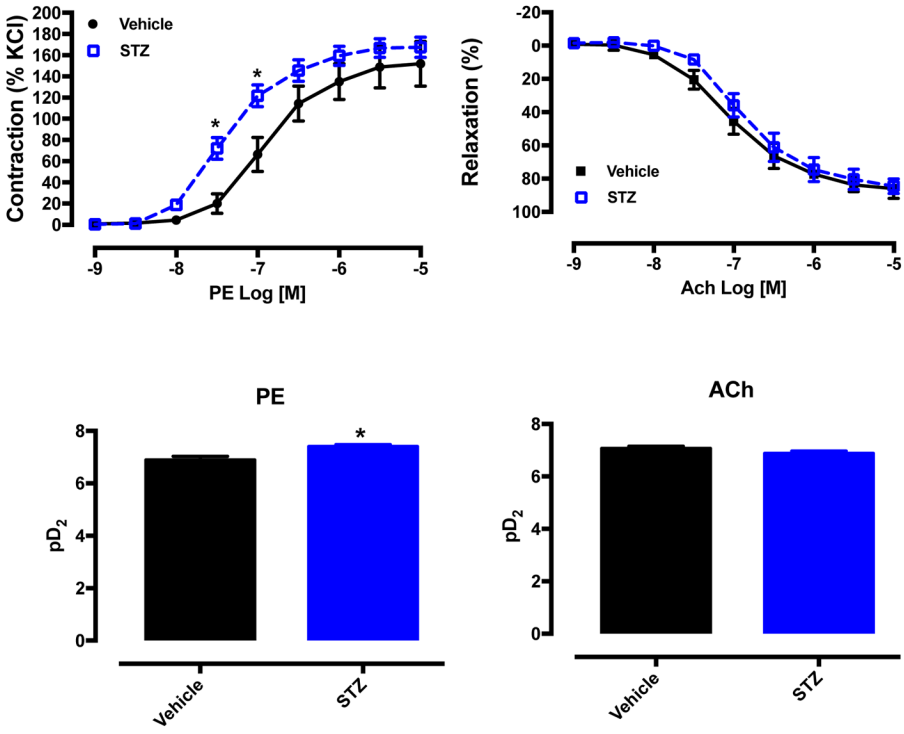

Fig. 3.

Aortas from diabetic mice exhibited an increased contraction to PE (left panels). CRCs to PE and Ach were performed in aortas isolated from vehicle- and STZ-treated mice (top). pD_2 values are presented for both PE and Ach (bottom panels). Data are represented as mean ± S.E.M. (n=5–7).*P< 0.05 vs vehicle.

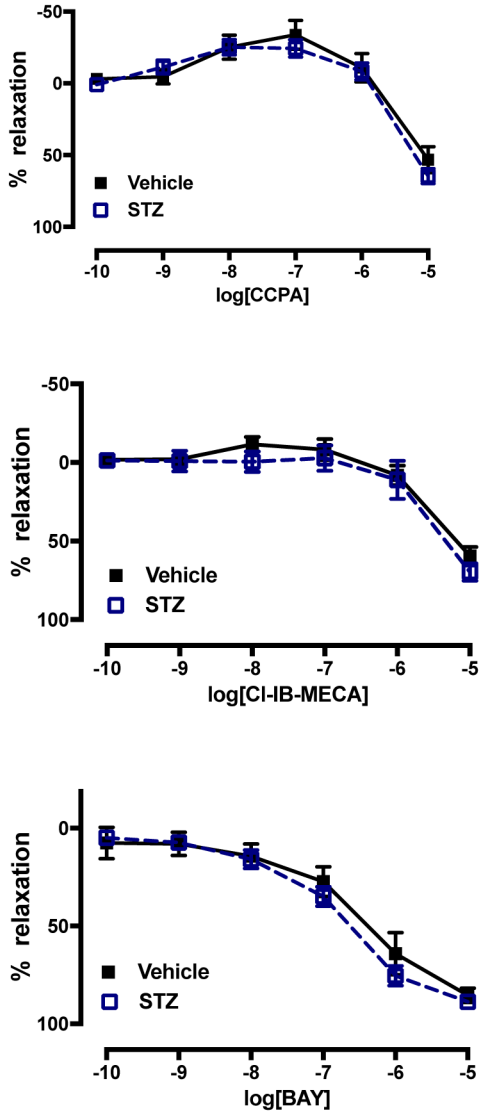

Fig. 4.

Diabetes did not affect resistance mesenteric artery responses to adenosine receptor(s) activation. CRCs to CCPA (A_1 agonist), Cl-IBMECA (A_3 agonist), and BAY (A_{2B} agonist) were performed in mesenteric arteries isolated from vehicle- and STZ-treated mice. Data are represented as mean ± S.E.M. (n=5–7).

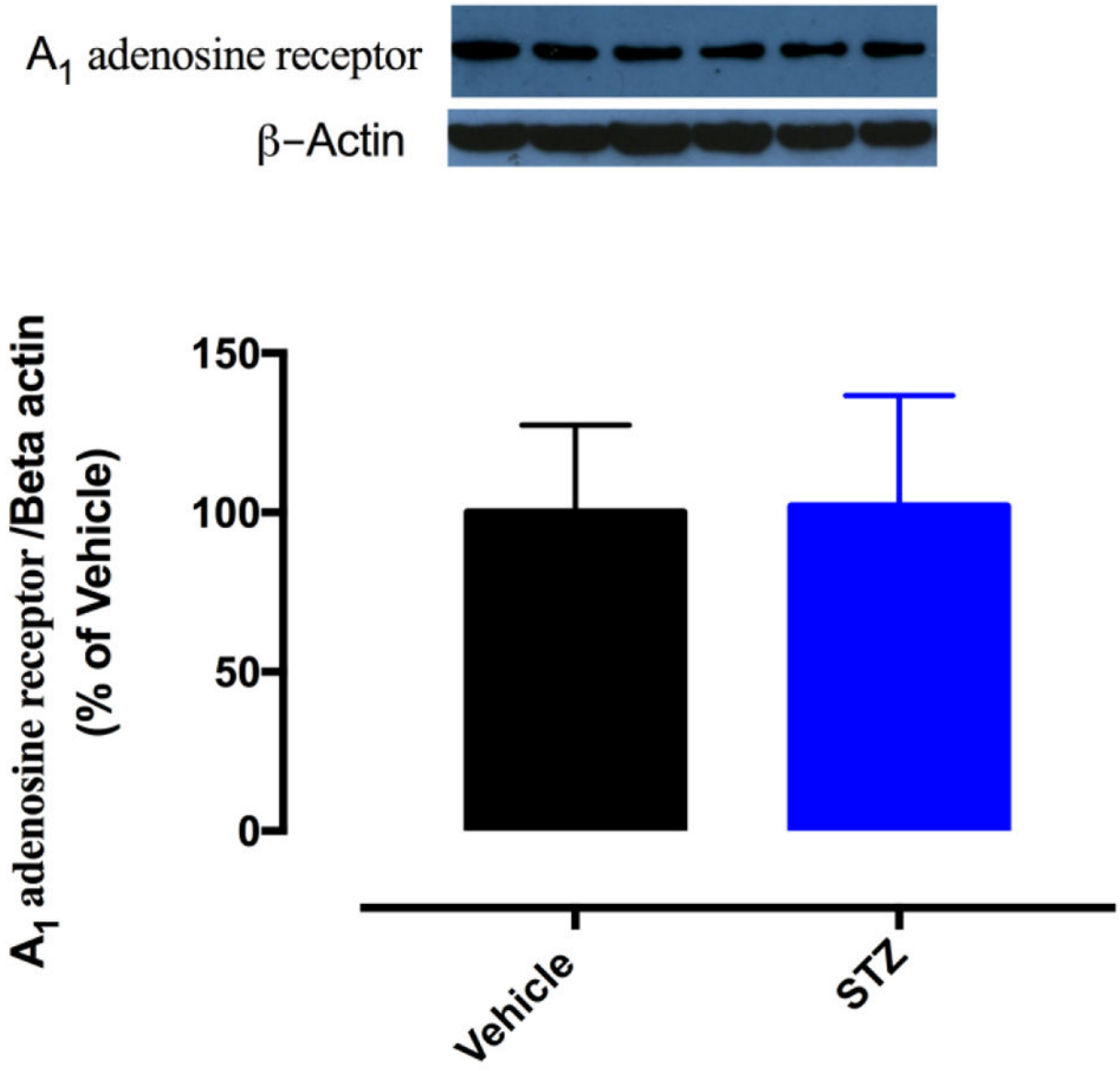

Fig. 5.

A_1 adenosine receptor expression in aortas isolated from vehicle- and STZ-treated mice was not different between the two groups. Results are represented as mean ± S.E.M. (n= 7–9).

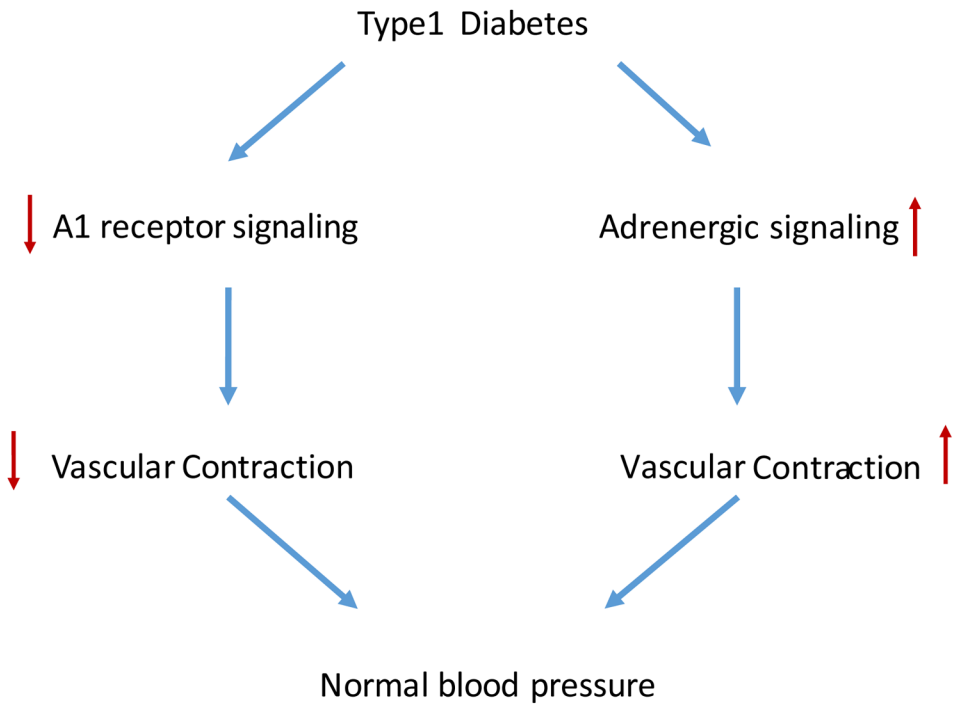

Fig. 6.

Possible mechanism of A_1 adenosine receptor contribution to the regulation of blood pressure in the early onset of T1D.