Gene deletion of the kinin receptor B1 attenuates cardiac inflammation and fibrosis during the development of experimental diabetic cardiomyopathy

Dirk Westermann\textsuperscript{1}, Thomas Walther\textsuperscript{1}, Konstantinos Savvatis\textsuperscript{1}, Meike Sobirey\textsuperscript{1}, Alexander Riad\textsuperscript{1}, Michael Bader\textsuperscript{2}, Heinz-Peter Schultheiss\textsuperscript{1}, Carsten Tschöpe\textsuperscript{1}

(1) Charité – Universitätsmedizin Berlin, Department of Cardiology and Pneumology, Campus Benjamin Franklin, Berlin, Germany;
(2) Max-Delbrück Center for Molecular Medicine, Berlin-Buch, Germany.

SHORT TITLE: Kinin Receptor Type 1 In Diabetic Cardiomyopathy

Corresponding author:
Carsten Tschöpe, MD,
E-mail: carsten.tschoepe@charite.de

Submitted 7 March 2008 and accepted 2 March 2009.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://diabetes.diabetesjournals.org.
Objective: Diabetic cardiomyopathy is associated with increased mortality in patients with diabetes mellitus. The underlying pathology of this disease is still under discussion. We studied the role of the kinin B1 receptor on the development of experimental diabetic cardiomyopathy.

Research Design and Methods: We utilized B1 receptor knockout mice and investigated cardiac inflammation, fibrosis and oxidative stress after induction of streptozotocin (STZ)-induced diabetes mellitus. Furthermore, the left ventricular function was measured by pressure-volume loops after 8 weeks of diabetes mellitus.

Results: B1 receptor knockout mice showed an attenuation of diabetic cardiomyopathy with improved systolic and diastolic function in comparison with diabetic control mice. This was associated with a decreased activation state of the MAP kinase p38, less oxidative stress as well as normalized cardiac inflammation, shown by fewer invading cells and, no increase in matrix metalloproteinase-9 as well as the chemokine CXCL-5. Furthermore, the pro-fibrotic connective tissue growth factor was normalized, leading to a reduction in cardiac fibrosis despite severe hyperglycemia in mice lacking the B1 receptor.

Conclusion: These findings suggest that the B1 receptor is detrimental in diabetic cardiomyopathy in that it mediates inflammatory and fibrotic processes. These insights might have useful implications on future studies utilizing B1 receptor antagonists for treatment of human diabetic cardiomyopathy.
Diabetic cardiomyopathy as it occurs in patients with diabetes mellitus carries a substantial risk concerning the subsequent development of heart failure and increased mortality (1). Different pathophysiological stimuli are involved in its development and mediate tissue injury leading to left ventricular systolic and diastolic dysfunction. Accumulation of cardiac fibrosis with distinct changes in the regulation of the extracellular matrix (2; 3), excessive generation of reactive oxygen species (4) and cardiac inflammation (5; 6), characterized by increased levels of pro-inflammatory cytokines and transendothelial migration of immunocompetent cells play a role in the manifestation of diabetic cardiomyopathy. Experimental stimulation of the local tissue kallikrein-kinin system has been shown to be beneficial in different forms of cardiomyopathies (7-11). Most of these effects are attributed to the kinin B2 receptor (B2R), while the role of the kinin B1 receptor (B1R) in cardiac failure is still under discussion. In contrast to the B2R, which is constitutively expressed in the cardiac tissue, the B1R is expressed at very low levels under basal conditions. Nevertheless, it is highly inducible under pathological conditions by pathological mediators such as bacterial lipopolysaccharide (12), cytokines (13), ischemia but also by hyperglycemia (14), as could be shown in different animal models of cardiomyopathy. Also in endomyocardial biopsies of patients with end-stage heart failure this up-regulation could be demonstrated and correlated with increased expression of pro-inflammatory cytokines in those patients (15). Whether B1R up-regulation is cardio-protective, parallel to that of the B2R (16; 17), or is cardio-toxic (13; 18; 19) remains debated. To further clarify the role of the B1R in the pathogenesis of diabetic cardiomyopathy, we investigated the LV function in an animal model of streptozotocin (STZ)-induced diabetes mellitus type-1 using B1R knockout mice. Furthermore, changes in the left ventricular remodeling, inflammation and oxidative stress were analyzed.

RESEARCH DESIGN and METHODS

Twenty-five B1R knockout mice (B1R-/-) on a C57/Bl6 genetic background and 25 littermates (B1R +/-) aged 2 months were obtained from the Max-Delbrück Center for Molecular Medicine, Berlin-Buch, Germany (13).

http://hyper.ahajournals.org/cgi/content/full/45/4/747 - R16-M043752

Diabetes mellitus was induced by injection of streptozotocin (STZ) (50 mg/kg i.p. for 5 days) in 15 B1R-/- (B1R-/--STZ) and in 15 C57/BL6 (B1R +/--STZ; while the others served as non-diabetic controls (B1R-/- and Con). Hyperglycaemia (glucose > 22 mmol/l) was confirmed 7 days later using a reflectance meter (Acutrend, Boehringer, Mannheim, Germany), as well as at the end of the study (glucose > 30 mmol/l). The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Surgical Procedures and Hemodynamic Measurements: Eight weeks after induction of diabetes mellitus, left ventricular (LV) function was analyzed using pressure-volume loops. The animals were anesthetized (thiopental 125 mg/g i.p.), intubated, and artificially ventilated. As described recently (20), a 1.2 F microconductance pressure catheter (SciSense, Ontario, Canada) was positioned in the LV for registration of LV pressure-volume (PV) loops in a closed-chest model. Indices of cardiac function were derived from PV data obtained both at steady state and during transient preload reduction by...
occlusion. Systolic function was quantified by LV end-systolic pressure (LVP, mmHg), dP/dt max (mmHg/s) and by ejection fraction (EF, %). Global cardiac function was quantified by the end systolic volume (ESV, µl), end diastolic volume (EDV, µl) stroke volume (SV, µl), cardiac output (CO, µl/min), the ratio of cardiac output / bodyweight (ml/min/g) and heart rate (HR, beat/min). Diastolic function was measured by LV end-diastolic pressure (LVEDP, mmHg), dP/dt min (mmHg/s), and the diastolic stiffness. Diastolic stiffness was calculated from the end diastolic pressure volume relationship (EDP=C*exp(b-Ved) with b for stiffness (21)). Moreover, mean arterial blood pressure was analyzed from measurements in the arteria carotis (22). Cardiac tissue was harvested and snap frozen for later measurements. All following measurements were performed in 10 animals per group.

**Histological measurements:** Immunohistochemistry was carried out using primary antibodies for collagen type 1, type 3, the connective tissue growth factor (CTGF), the matrix-metalloproteinase-9 (MMP-9), CD3⁺, CD11b⁺, CD45⁺, CD68⁺ cells as well as nitrotyrosine and myeloperoxidase (MPO) (all from Serotec, Munich, Germany) followed by the DAKO Envision HRP technique (DAKO, Glostrup, Denmark). Histological costainings were performed using primary antibodies for CD68, TNF-alpha (R&D Systems, Wiesbaden, Germany), sarcomeric actin as well as TAB-1 (Cell Signaling Technology, Danvers, MA, USA).

**Realtime RT PCR:** Realtime RT-PCR (ABI PRISM 7900 HT Sequence Detection System software, version 2.2.2.; Perkin Elmer) was carried out as described previously (23) using primers for the kinin B1 and B2 receptor, the cytokine interleukin 1-beta (IL-1β), tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) as well as for the chemokine CXCL-5. 18S was used as housekeeping gene.

**Western blot for evaluation of total p38 MAPK and p38 phosphorylation:** Total p38 MAPK and phosphorylated p38 MAPK were detected with each specific antibody. Moreover, TAB-1 (all from Cell Signaling Technology, Danvers, MA, USA) was detected. The blots were visualised with a chemiluminescence system (Amersham Bioscience, Buckinghamshire, UK). Quantitative analysis of the intensity of the bands was performed with NIH Image 1.63 software (National Institutes of Health, Bethesda, MD, USA).

**Statistical Analysis:** All data are expressed as mean ± SEM. Statistical significance between multiple groups was determined using ANOVA and posthoc analysis with a Bonferroni test. Values of p<0.05 were considered significant.

**RESULTS**

Eight weeks after induction of STZ induced diabetes mellitus, glucose levels were found to be highly increased in B1R⁻/⁻-STZ and B1R⁺/⁺-STZ, but did not differ between both diabetic groups. Body weight decreased in both groups when compared to controls (Table 1).

**Hemodynamic data:** Lack of B1R had no effect on cardiac function under normoglycaemic conditions. The heart rate was significantly decreased in B1R⁺/⁺-STZ when compared to controls, due to the known effect of diabetic cardiac autonomy (24), while B1R⁻/⁻-STZ were not statistically different from their controls. No ventricular dilatation was demonstrated in either STZ group when compared to controls (Table 1).

**Western blot for evaluation of total p38 MAPK and p38 phosphorylation:** Total p38 MAPK and phosphorylated p38 MAPK were detected with each specific antibody. Moreover, TAB-1 (all from Cell Signaling Technology, Danvers, MA, USA) was detected. The blots were visualised with a chemiluminescence system (Amersham Bioscience, Buckinghamshire, UK). Quantitative analysis of the intensity of the bands was performed with NIH Image 1.63 software (National Institutes of Health, Bethesda, MD, USA).

**Statistical Analysis:** All data are expressed as mean ± SEM. Statistical significance between multiple groups was determined using ANOVA and posthoc analysis with a Bonferroni test. Values of p<0.05 were considered significant.

**RESULTS**

Eight weeks after induction of STZ induced diabetes mellitus, glucose levels were found to be highly increased in B1R⁻/⁻-STZ and B1R⁺/⁺-STZ, but did not differ between both diabetic groups. Body weight decreased in both groups when compared to controls (Table 1).

**Hemodynamic data:** Lack of B1R had no effect on cardiac function under normoglycaemic conditions. The heart rate was significantly decreased in B1R⁺/⁺-STZ when compared to controls, due to the known effect of diabetic cardiac autonomy (24), while B1R⁻/⁻-STZ were not statistically different from their controls. No ventricular dilatation was demonstrated in either STZ group when compared to controls (Table 1).

**Statistical Analysis:** All data are expressed as mean ± SEM. Statistical significance between multiple groups was determined using ANOVA and posthoc analysis with a Bonferroni test. Values of p<0.05 were considered significant.
decreased, the end diastolic pressure as well as diastolic stiffness were increased when STZ was compared to the control group. However, the impairment in these parameters was much less pronounced in B1R$^{-/-}$-STZ (Table 1 and Figure 1).

**Cardiac inflammation and oxidative stress:** In the myocardium of the diabetic mice, the mRNA abundance of the pro inflammatory cytokines IL-1$\beta$, IL-6 and TNF-alpha as well as the chemokine CXCL-5 were significantly increased by B1R$^{+/+}$-STZ compared to controls (Figure 2). This was associated with increased numbers of CD3$, CD11b$, CD45$, CD68$^+$ cells (Figure 3) and the protein abundance of MMP-9 (Figure 4). This up-regulation was prevented by B1R$^{-/-}$-STZ resulting in normalized levels compared to controls. Moreover, a major part of TNF-alpha is produced by inflammatory cells (CD68) (Figure 5). The abundance of nitrotyrosine and myeloperoxidase was increased in the cardiac tissue of the STZ group, as an indicator of increased oxidative stress (Figure 4). The lack of the B1R reduced this increased expression of nitrotyrosine and myeloperoxidase in comparison with STZ, despite severe hyperglycemia when B1R$^{-/-}$-STZ was compared to B1R$^{+/+}$-STZ. (Figure 4)

Protein levels of endothelial NO synthase levels (eNOS) were down regulated in both diabetic groups. Nevertheless, the mRNA content was only down regulated significantly in the the B1R$^{+/+}$-STZ but not in the B1R$^{-/-}$-STZ. (Figure 4)

Furthermore, the phosphorylation state of the MAP kinase p38, known to contribute to tissue inflammation was increased in STZ compared to controls, again an effect which was reduced in B1R$^{-/-}$-STZ when compared to controls. Moreover, TAB-1 protein was significantly increased in diabetic wildtypes compared to B1R$^{-/-}$-STZ. (Figure 6)

**Kinin receptor regulation:** The B1 receptor mRNA was increased by STZ induced diabetes mellitus in the wildtypes. The B2R mRNA was also increased due to diabetic condition in the wildtypes. In B1R$^{-/-}$ animals the B2 receptor expression was higher compared to the wildtypes under basal conditions. Interestingly, there was no further B2 mRNA up regulation due to diabetic conditions as seen in the wildtypes. (Figure 2)

**Cardiac fibrosis:** CTGF was highly increased in the diabetic animals. This increase in CTGF was accompanied by increased levels of collagen type 1 and 3, as an indicator of cardiac fibrosis. In contrast, CTGF was normalized in B1R$^{-/-}$-STZ, which translated into normalized collagen type 1 and type 3 levels as well, when compared to the controls. (Figure 7)

**DISCUSSION**

The salient finding of this study is that B1R gene deletion attenuates cardiac systolic and diastolic dysfunction in experimental diabetic cardiomyopathy. Diabetic cardiomyopathy is characterized by an increase in the phosphorylation state of the MAP kinase p38, which was associated with augmented cardiac inflammation, cardiac fibrosis and oxidative stress in the cardiac tissue. These changes were normalized in mice lacking the B1R despite the occurrence of comparable severe hyperglycaemia.

Experimental stimulation of the kallikrein-kinin system by gene transfer (25; 26) and/or by the use of transgenic kallikrein over-expressing animals (10) attenuates diabetic cardiomyopathy. This is in agreement with other studies which showing potent cardio-protective effects of the kallikrein-kinin system in animal models of ischemic (27-29), pressure overload (8), septic (12) and hypertensive (9) cardiomyopathy. We and others, using the STZ model of diabetes mellitus, were able to show that these cardio beneficial effects of the kallikrein-kinin mediate anti-inflammatory and anti-fibrotic effects and furthermore reduce oxidative stress (10; 30; 31) as well as improve glucose
utilization and lipid metabolism (26; 32). Both receptors of the system, the B1R and the B2R, are up-regulated in the diabetic heart (14). The cardio protective effects are mediated mainly by the B2R, since pharmacological inhibition of the B2R was seen to abolish these cardio-protective effects (31). The relationship between B1R and the development of heart failure is still under investigation. Recently, it was shown that the B1R may yield similar effects when compared to the B2R in an animal model of myocardial infarction (17). Nonetheless, other researchers have shown that a lack of the B1R reduced infarct size in ischemia reperfusion injury (19; 33), a finding which indeed may imply an opposite function compared to that of the B2R. While there is good evidence that the B1R plays a detrimental role in autonomic diabetic nociception (34), obstructive nephropathy (35) and stroke (36) by modulating inflammatory processes and increasing inflammation, its role in the development of diabetic cardiomyopathy has not yet been directly investigated.

We demonstrated recently that diabetic cardiomyopathy is associated with increased cardiac inflammation (2; 5; 10). These inflammatory processes were associated with increased oxidative stress and cardiac fibrosis, all contributing to systolic and diastolic dysfunction under diabetic conditions. Since the B1R is known to be up-regulated by IL-1β (37) and hyperglycemia (14; 38) and mediate tissue inflammation by increasing invading cells and pro-inflammatory cytokines in airway diseases (39), we investigated its role during the development of diabetic cardiomyopathy. On the one hand, in non-diabetic mice with gene deletion of the B1R, the cardiac function remained unchanged, when this was compared to control mice in the current study. That is a finding which is in agreement with others (17; 40-42). On the other hand, attenuated cardiac dysfunction despite severe hyperglycaemia indicates a detrimental role of the B1R in diabetic cardiomyopathy, when systolic and diastolic function of B1R−/−-STZ were compared to STZ.

One intracellular pathway of the B1R was shown to be dependent on the MAP kinase p38 (43). The current study shows an activation of the p38 pathway in the STZ group, which was normalized in the B1R−/−-STZ group. P38 phosphorylation can be induced by TAB-1 (44). In line with these findings, increased protein content of TAB-1 in diabetic wildtypes compared to B1R−/−-STZ could be documented here. Since p38 activation plays a role in diabetic cardiomyopathy by inducing cardiac inflammation (45), we documented increased cardiac levels of cytokines in the STZ group, namely of IL-1β, IL-6 and TNF-alpha, known to cause myocardial dysfunction and mediate leukocyte infiltration during tissue inflammation (46). This cytokine induction was decreased in diabetic mice lacking the B1R.

Moreover, the number of invading immunocompetent cells was increased in the STZ group, thus yielding another marker of cardiac inflammation. These increased numbers were normalized in mice lacking the B1R. This is important, since those invading cells are one major source of cytokine production within the cardiac tissue, e.g. as shown here by the colocalization of TNF-alpha with CD68+ cells. Furthermore, we show that the protein levels of MMP-9 were also increased in STZ and decreased in diabetic mice with gene deletion of the B1R. Recent findings suggest that especially MMP-9 modulates the transendothelial migration of leucocytes from the vessel to the tissue, where an inflammatory process is ongoing (47). This can be explained by the fact that MMP-9 does not solely cleave gelatine, but indeed processes and activates many chemokines and cytokines and thereby directly modulates inflammation. In line with these effects, it was
shown in a mice model of hepatitis that MMP-9 knockout mice were protected against invading leucocytes undergoing transendothelial migration (48). Only recently it could be shown that induction of MMP-9 expression is triggered by bradykinin, using cell culture rat astrocytes (49). Although those authors showed that B2R antagonism inhibited this increase (49), our data suggest that the B1R plays a role in MMP-9 expression in cardiac tissue under diabetic conditions, also. Moreover, the B1R was shown to be essential for IL-1β driven cell recruitment of immunocompetent cells by inducing the chemokine CXCL5 in endothelial cells, recently (50). This recruitment of CXCL-5, known to be one important player in leucocytes recruitment to sites of tissue inflammation, was abolished in mice without the B1R or when a pharmacological B1R antagonist was applied (50). In line with these data, we show here that the chemokine CXCL-5 was increased under diabetic conditions. This effect was completely normalized in mice lacking the B1R despite severe hyperglycemia. This normalized levels of CXCL-5 and MMP-9 result in reduced migration of inflammatory cells into the cardiac tissue. Therefore, inflammation leading to cardiac damage due to invasion of these cells was attenuated in diabetic B1R−/−-STZ compared to diabetic controls with increased levels of CXCL-5 and MMP-9.

Furthermore, it was shown that the B1R, but not the B2R increases the mRNA abundance of the pro-fibrotic CTGF and thereby increases collagen mRNA and protein production in human fibroblasts, an effect which could be blocked by a B1R antagonist (51). Following these findings, we show increased levels of CTGF in the cardiac tissue of STZ mice leading to increased collagen accumulation, which is known to be a hallmarks in the development of diabetic cardiomyopathy leading to increased cardiac stiffness contributing to diastolic and systolic failure. Together with those findings, this mechanism could not be observed in mice with B1R gene deletion, thus showing no increase in CTGF or collagen accumulation.

Much evidence has indicated that oxidative stress plays an important role in the failing diabetic heart (52). This can be attenuated by the kallikrein-kinin system (8; 10). Consistently, we show increased nitrotyrosine and myeloperoxidase protein levels (expressed from inflammatory cells) in the cardiac tissue of the STZ group. Together with reduced inflammatory cells, nitrotyrosine and myeloperoxidase were reduced in the B1R−/−-STZ group. These data suggest that the B1R might play a role in the generation of oxidative stress, most probably due to the increased recruitment of inflammatory cells resulting in increased myeloperoxidase present in the cardiac tissue. Future studies have to investigate whether this effect is only mediated by the B1R, or if changes in basal B2R level expression – as shown in this study - are also influencing generation of oxidative stress in the B1R−/−-STZ group.

The protein content of eNOS, known to exert antioxidative effects, was similarly reduced in both diabetic groups. Interestingly, B1R−/−-STZ showed no significant down regulation on mRNA levels. Despite these changes in mRNA content, which may be explained by post transcriptional modification, these data suggest that the diabetes induced down regulation of eNOS protein is not mainly regulated by the B1R knockout. Again, further studies have to reveal the impact of B2R regulation on these effects.

In conclusion, this study demonstrates that a lack of the B1R attenuates the development of STZ-induced diabetic cardiomyopathy with a decrease of cardiac inflammation, fibrosis and oxidative stress.

ACKNOWLEDGEMENT
This study was supported by the Deutsche Forschungsgemeinschaft (SFB-TR-19; A2, Z3). The authors would like to thank M. Kastner for proof-reading the final version of the manuscript.

**Figure Legends**

**Figure 1:** Representative pressure volume loops during a preload reduction of control mice (B1R +/+ ) or mice lacking the B1R (B1R -/- ) under basal conditions and 8 weeks after induction of streptozotocin (STZ)-induced diabetes mellitus. The thick black line at the bottom indicates the left ventricular stiffness, which is increased in B1R +/+ STZ (indicated by the thick black arrow).

**Figure 2:** MRNA levels of cardiac cytokines in control mice (B1R +/+ ) or mice lacking the B1R (B1R -/- ) under basal conditions and 8 weeks after induction of streptozotocin (STZ)-induced diabetes mellitus with increased levels of interleukin 1-beta (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) in STZ measured by realtime RT-PCR. Moreover, mRNA levels of the chemokine CXCL-5 levels as well as mRNA levels of the B1 and B2 receptor.

* = p<0.05 vs. B1R +/+ and B1R -/- STZ. # = p<0.05 vs. B1R +/+ 

**Figure 3:** Increased inflammatory cells (CD3+, CD11+, CD45+ and CD68+) in the cardiac tissue of B1R +/+ -STZ with representative pictures of all groups for CD11+ and CD68+ cells in control mice (B1R +/+ ) or mice lacking the B1R (B1R -/- ) under basal conditions and 8 weeks after induction of streptozotocin (STZ)-induced diabetes mellitus.

* = p<0.05 vs. B1R +/+ and B1R -/- STZ

**Figure 4:** Increased protein levels of oxidative stress (nitrotyrosin and myeloperoxidase (MPO)) as well as the matrix metalloproteinase-9 (MMP-9) in the cardiac tissue of B1R +/+ -STZ. Moreover, protein levels and mRNA levels of the endothelial NO synthase (eNOS). Representative pictures of all groups for MPO and eNOS.

* = p<0.05 vs. B1R +/+ 

**Figure 5:** Immunofluorescent stainings of cardiac tissue of a diabetic wildtype mouse with stainings for CD68, TNF-alpha as well alpha sacromeric actin and DAPI (for cell nuclei) showing that TNF-alpha is secreted by inflammatory CD68+ cells.

**Figure 6:** Panel A: Immunofluorescent stainings of cardiac tissue of B1R +/+ -STZ and B1R -/- STZ showing protein levels of TAK-1 binding protein (TAB-1) and alpha sacromeric actin as well as DAPI (for cell nuclei). This demonstrates a reduced protein content of TAB-1 in B1R -/- STZ. Panel B: Quantification of protein levels of the MAP kinase P38 and its phosphorylated form as well as TAB-1 showing a normalization of the p38 activation and the TAB-1 protein content in B1R -/- STZ compared to B1R +/+ -STZ.
* = p<0.05 vs. B1R +/- STZ.

**Figure 7:** Increased levels of connective tissue growth factor (CTGF) and collagen type I and III in cardiac tissue of B1R +/- STZ compared to non diabetic controls. B1R +/- STZ have normalized cardiac fibrosis as well as normalized levels of CTGF.

* = p<0.05 vs. B1R ++/++ and B1R +/- STZ.
REFERENCES


46. Kristiansen OP, Mandrup-Poulsen T: Interleukin-6 and diabetes: the good, the bad, or the indifferent? *Diabetes* 54 Suppl 2:S114-124, 2005
### Table 1:

<table>
<thead>
<tr>
<th></th>
<th>B1R +/+</th>
<th>B1R +/-</th>
<th>B1R +/+ STZ</th>
<th>B1R +/- STZ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight [g]</strong></td>
<td>27±1</td>
<td>28±1</td>
<td>17±1*</td>
<td>17±1*</td>
</tr>
<tr>
<td><strong>Glucose levels [mmol/L]</strong></td>
<td>6±0.2</td>
<td>6±0.2</td>
<td>31±2*</td>
<td>32±2*</td>
</tr>
<tr>
<td><strong>Hemodynamic function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Heart rate [b/min]</strong></td>
<td>465±22</td>
<td>486±32</td>
<td>302±27*</td>
<td>389±35</td>
</tr>
<tr>
<td><strong>Volume end diastolic [µl]</strong></td>
<td>53±2</td>
<td>48±4</td>
<td>49±4</td>
<td>47±3</td>
</tr>
<tr>
<td><strong>Volume end systolic [µl]</strong></td>
<td>18±4</td>
<td>15±3</td>
<td>28±5</td>
<td>23±5</td>
</tr>
<tr>
<td><strong>stroke volume [µl]</strong></td>
<td>35±6</td>
<td>31±4</td>
<td>19±6*</td>
<td>24±5</td>
</tr>
<tr>
<td><strong>cardiac output [ml/min]</strong></td>
<td>16.1±1</td>
<td>15.8±1</td>
<td>6.3±0.6*</td>
<td>9.3±1&quot;</td>
</tr>
<tr>
<td><strong>Cardiac output / bodyweight [ml/min/g]</strong></td>
<td>0.61±0.02</td>
<td>0.57±0.04</td>
<td>0.41±0.04*</td>
<td>0.56±0.03</td>
</tr>
<tr>
<td><strong>LV systolic pressure [mmHg]</strong></td>
<td>98±4</td>
<td>103±5</td>
<td>72±5*</td>
<td>86±6&quot;</td>
</tr>
<tr>
<td><strong>dP/dt max [mmHg/s]</strong></td>
<td>6658±346</td>
<td>6858±256</td>
<td>3215±201*</td>
<td>5214±286&quot;</td>
</tr>
<tr>
<td><strong>Ejection Fraction [%]</strong></td>
<td>66±4</td>
<td>64±5</td>
<td>38±7*</td>
<td>51±4&quot;</td>
</tr>
<tr>
<td><strong>LV diastolic pressure [mmHg]</strong></td>
<td>2.5±1</td>
<td>2.8±1</td>
<td>9.4±2*</td>
<td>4.4±2&quot;</td>
</tr>
<tr>
<td><strong>dP/dt min [mmHg/s]</strong></td>
<td>-5896±301</td>
<td>-5485±285</td>
<td>-2248±247*</td>
<td>-4257±244&quot;*</td>
</tr>
<tr>
<td><strong>LV stiffness [ml⁻¹]</strong></td>
<td>0.027±0.002</td>
<td>0.034±0.002</td>
<td>0.127±0.001*</td>
<td>0.047±0.004&quot;*</td>
</tr>
<tr>
<td><strong>Mean blood pressure [mmHg]</strong></td>
<td>95±4</td>
<td>98±5</td>
<td>68±7*</td>
<td>88±6&quot;</td>
</tr>
</tbody>
</table>

Hemodynamic function of control mice (B1R +/+ ) or mice lacking the B1R (B1R +/- ) under basal conditions and 8 weeks after induction of streptozotocin (STZ)-induced diabetes mellitus. Left ventricular (LV), Contractility (dP/dt max), relaxation (dP/dt min); significances indicated by * = p<0.05 vs. non diabetic controls and by # = p<0.05 vs. B1R +/+ STZ.
Figure 1:
Figure 2:

- **IL1-β**
- **IL6**
- **TNF-alpha**
- **CXCL-5**
- **B1 receptor**
- **B2 receptor**
Figure 3:

**CD3**\(^+\) cells

- B1\(^+\+)
- B1\(^-\)
- B1\(^+\) + STZ
- B1\(^-\) + STZ

**CD11**\(^+\) cells

- B1\(^+\+)
- B1\(^-\)
- B1\(^+\) + STZ
- B1\(^-\) + STZ

**CD45**\(^+\) cells

- B1\(^+\+)
- B1\(^-\)
- B1\(^+\) + STZ
- B1\(^-\) + STZ

**CD68**\(^+\) cells

- B1\(^+\+)
- B1\(^-\)
- B1\(^+\) + STZ
- B1\(^-\) + STZ

**CD11**\(^+\) cells

- B1-Co\(^+\+)
- B1-STZ\(^+\+)
- B1-Co\(^-\)
- B1-STZ\(^-\)

**CD68**\(^+\) cells

- B1-Co\(^+\+)
- B1-STZ\(^+\+)
- B1-Co\(^-\)
- B1-STZ\(^-\)
Figure 4:
Figure 7: