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Article

The Effects of Low-Dose Empagliflozin on Cardiac Function and B-Adrenoceptor Responses in a Rat Model of Streptozotocin-Induced Diabetes

Zeynep E. Yesilyurt[1], Irem Karaomerlioglu[2], Betul R. Erdogan[3], Gaye Ozturk[4], Martin C. Michel[5*], Ebru Arioglu-Inan[6]

[1] Dept. of Pharmacology, Ankara University, Ankara, Turkey; Dept. of Pharmacology Gazi University, Ankara, Turkey; zeynepelifyesilyurt@gazi.edu.tr
[2] Dept. of Pharmacology, Ankara University, Ankara, Turkey; Turkish Medicines and Medical Devices Agency; irem.karaomerlioglu@gmail.com
[3] Dept. of Pharmacology, Ankara University, Ankara, Turkey; Dept. of Pharmacology, Izmir Katip Celebi University, Izmir, Turkey; betulrabia.erdogan@ikcu.edu.tr
[4] Dept. of Pharmacology, Hacettepe University, Ankara, Turkey; gaye_ozturk@hotmail.com.tr
[5] Dept. of Pharmacology, University Medical Center, Johannes Gutenberg University, Mainz, Germany; marmiche@uni-mainz.de
[6] Dept. of Pharmacology, Ankara University, Ankara, Turkey, arioglu@ankara.edu.tr
* Correspondence: marmiche@uni-mainz.de; Tel.: +49-6131-179346

Abstract: Diabetes mellitus leads to cardiovascular complications including impaired cardiac β-adrenoceptor (β-AR) function. Sodium-glucose cotransporter-2 (SGLT2) inhibitors, such as empagliflozin (EMPA) improve outcomes in heart failure patients and animal models thereof. Therefore, we have investigated the effects of EMPA on *in vivo* cardiac function (pressure-volume loop analysis) and β-AR-mediated contractile responses (papillary strips) in streptozotocin (STZ; 40 mg/kg, i.p.)-induced diabes in male Sprague Dawley rats (control, EMPA-treated control, diabetic, EMPA-treated diabetic) in a design reflecting late-onset treatment. 13-16 weeks after STZ injection treatment with a low dose of EMPA (10 mg/kg/day, daily oral gavage) or vehicle was administered for another 8 weeks. EMPA did not change cardiac function in control rats. Diabetic rats had a reduced heart rate, cardiac output, stroke work, rate of contration and rate of relaxation and increased isovolumic relaxation, whereas *in vitro* responses were not markedly attenuated. Treatment with EMPA showed a trend for improvement of some but not all parameters. Our results indicate that low dose EMPA treatment had limited effects on cardiac impairment despite reducing blood glucose when initiated after diabetes is manifest. Future studies with a higher dose and greater sample sizes could help to clarify the possible benefits of EMPA on the diabetic heart.

Keywords: β-adrenoceptor; diabetes; empagliflozin; heart; pressure-volume loop analysis

1. Introduction

Diabetes remains an important health problem despite the steps taken to better understand its pathophysiology and new treatment approaches [1]. The International Diabetes Federation estimates that it affected 451 million people worldwide in 2017 and will affect 693 million by 2045 [2]. Cardiovascular complications are one of the most important causes of morbidity and mortality in patients with type 1 and type 2 diabetes (T1DM and T2DM, respectively) [3]. While a good glycemic control is important to prevent microvascular diabetic complications such as nephropathy, retinopathy and neuropathy, the ACCORD [4], ADVANCE [5] and VADT [6] trials have shown that major cardiovascular events, considered to represent macrovascular complications, were inadequately addressed by treatment approaches despite a tight glycemic control.

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Inhibitors of the sodium-glucose cotransporter-2 (SGLT2) are antidiabetic drugs that inhibit glucose reabsorption in the renal tubules to stimulate glucose excretion [7]. The cardiovascular outcome studies of empagliflozin (EMPA) [8], canagliflozin [9], dapagliflozin [10] and ertugliflozin [11] have shown that diabetic patients treated with SGLT2 inhibitors had a lower risk of major cardiovascular events compared with placebo including a reduced rate of hospitalization for heart failure and lower all-cause mortality. These studies sparked additional trials designed to study effects on heart failure that similarly included diabetic and non-diabetic patients, either in heart failure with reduced ejection fraction [12,13] or, more recently, with preserved ejection fraction [14]. SGLT2 inhibitors effectively reduced heart failure endpoints in such studies, and that effect was similarly pronounced in heart failure patients with and without concomitant diabetes. Accordingly, SGLT2 inhibitors are now recommended by major guidelines for the routine management of heart failure patients [15,16].

The effects of SGLT2 inhibitors have been explored in various rodent models of diabetes-associated cardiomyopathy. These have used T2DM models including ob/ob mice [17,18], db/db mice with or without additional angiotensin II infusion [19,20], rats [21-23] and mice [24] treated with a combination of a high-fat diet (HFD) and injection of streptozotocin (STZ), Zucker diabetic fatty rats [25], seipin knock-out mice [26], a cross-breed of mRen27 and Tet29 rats [27], and Dahl salt-sensitive rats on a high-sodium diet [28]. They have used various SGLT2 inhibitors including canagliflozin, dapagliflozin and EMPA. While SGLT2 inhibitors are only indicated for the treatment of T2DM, some studies have also tested them in animal models of T1DM, i.e., rats and, less frequently, mice injected with STZ [29-34]. These studies generally confirmed the clinically observed beneficial effect on diabetic cardiomyopathy.

Despite the compelling evidence for the use of SGLT2 inhibitors in the treatment of heart failure, the underlying mechanisms remain to be understood. Given that SGLT2 inhibitors are similarly effective in the treatment of heart failure in patients with and without diabetes [12,13], their beneficial effects on heart failure apparently are not a direct consequence of their glucose lowering effects. While the clinical studies yielded limited mechanistic explanation of the beneficial effects, the above rodent studies have proposed a range of potential molecular and cellular mechanisms that could contribute including those related to the inflammasome, fibroblast activation or activation of various protein kinases. The probably most consistently observed cellular and molecular phenotype in heart failure in general is a decreased responsiveness to β-adrenoceptor (β-AR) stimulation that is at least partly due to down-regulation of the receptor [35-38]. Both desensitization and down-regulation primarily affect β_1-AR, whereas β_2-AR are less consistently affected, and β_3-AR even are up-regulated in most studies [39]. Animal studies show that desensitization and down-regulation of β-AR, particularly β_1-AR, also occurs in the diabetic heart, e.g., in rats injected with STZ [40,41] or in Zucker diabetic fatty rats [42]. Surprisingly, to the best of our knowledge, the effect of SGLT2 inhibitors on diabetic cardiomyopathy on reduced β-AR responses has not been investigated. Therefore, the present pilot study was designed to explore effects of EMPA as example for SGLT2 inhibitors in general on β-AR responses as assessed in isolated papillary muscle in a rat model of STZ-induced diabetes; effects on general cardiac function assessed by pressure-volume (PV) loop analysis were also measured. While our findings recapitulate the known cardiac phenotype in the STZ model of T1DM, effect sizes were smaller than in several other studies. Moreover, in contrast to various other studies, EMPA had only small if any effects. While this could partly be due to limited effect sizes for STZ in the present study, we also propose a novel and testable hypothesis to explain this.

2. Materials and Methods

2.1. Animals and treatments

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Male, Sprague Dawley rats (11-12 weeks old) were obtained from Bilkent University, Department of Molecular Biology and Genetics Animal Unit (Ankara, Turkey) and housed under 12-h light/dark cycle in the Ankara University Faculty of Pharmacy Animal Care Unit. Rats had free access to standard rat chow (Purina Rat Chow; Optima AS, Bolu, Turkey) and tap water. All experimental procedures of the present study were in line with NIH guidelines for the care and use of experimental animals and had been approved by the Animal Welfare Committee of Ankara University (2019-4-41).

After one week of acclimatization, rats were randomly divided into control and diabetic groups. We set the sample sizes as 14 rats for the control group and 20 rats for the diabetic groups to account for possible attrition in the diabetic groups because of a greater mortality risk. Diabetes was induced by an intraperitoneal injection of STZ (40 mg/kg dissolved in citrate buffer at pH 4.5). Control rats were injected with citrate buffer (pH 4.5). Three days after STZ injection, blood glucose level was measured and rats with blood glucose levels higher than 300 mg/dl were considered as diabetic. If blood glucose levels were lower than 300 mg/dl, a second or third STZ injection was administered.

Control and diabetic rats were randomly divided into 2 groups each to yield control, EMPA treated control, diabetic and EMPA treated diabetic groups. One rat in the diabetic group was euthanized due to poor health condition in this period. Thus, the sample size became 7 in each control group, 8 in diabetic and 11 in EMPA treated diabetic group. The sample size was higher in the treated diabetic group regarding the possible mortality risk. The treatment was started at 13-16 weeks after STZ injection. For this purpose, EMPA (Jardiance®) tablets were crushed and suspended in distilled water. Each tablet includes lactose monohydrate, microcrystalline cellulose, hydroxypropyl cellulose, croscarmellose sodium, colloidal anhydrous silica, magnesium stearate. The film coating of the tablets also contains hypromellose, titanium dioxide, talc, macrogol, and yellow ferric oxide. The crushed material was dissolved in 5 ml of distilled water. Treated control and diabetic rats received 10 mg/kg EMPA (calculated for active pharmaceutical ingredient) by once daily oral gavage for 8 weeks. The dose was selected based on previous studies, where the lower of two doses under investigation was 10 mg/kg/day [43-45]. Control and diabetic rats received distilled water by oral gavage. In the treatment period, one rat from the EMPA treated control group was killed during treatment with gavage. One rat in the diabetic group returned to normoglycemic level and was excluded from the study. Two rats in the EMPA treated diabetic group were euthanized due to a poor health profile. At the end of the treatment period, rats were sacrificed, and *in vivo* and *in vitro* experiments were performed (control n=7, EMPA treated control n=6, diabetic n=7 and EMPA treated diabetic n=9).

2.2. In vivo measurement of basal hemodynamic parameters

PV loop analysis was performed as previously reported [46]. Rats were anesthetized by 2% isoflurane inhalation and body temperature was monitored to be 37°C with a rectal probe during the operation. After making an incision, a PV catheter (Transonic, London, Ontario, Canada) was inserted into right carotid artery. After 3-minute of blood pressure recording, the catheter was advanced into the left ventricle. Following a 10-minute stabilization period, PV loops were recorded. Then, preload-independent cardiac parameters were measured by 5-second *vena cava* occlusions. At the end of the experiments, the following basal hemodynamic parameters were calculated; end-diastolic pressure, end-systolic pressure, heart rate, end-diastolic volume, end-systolic volume, cardiac output, ejection fraction, stroke volume, stroke work, rate of contraction, rate of relaxation, isovolumic relaxation constant (Tau logistic) and preload independent parameters; preload recruitable stroke work, end-systolic pressure-volume relationship, end-diastolic pressure-volume relationship. End-systolic volume, end-diastolic volume, stroke volume and cardiac output were normalized to body weight to eliminate the differences of body weight between animals (end-systolic volume index, end-diastolic volume index, stroke volume index, cardiac index) [47].

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2.3. In vitro papillary muscle experiments

The experiments were performed as previously described [46]. Briefly, rats were anesthetized under 2% isoflurane inhalation. After PV loop analysis, hearts were rapidly isolated. The extracted hearts were transferred to an oxygenated experimental plate containing heparin and Krebs solution (120 mM NaCl; 4.8 mM KCl; 1.25 mM $CaCl_2.2H_2O$; 1.25 mM $MgSO_4.7H_2O$; 1.2 mM KH_2PO_4; 25 mM $NaHCO_3$ and 10 mM glucose at pH 7.4). Papillary muscles were dissected from the left ventricle and mounted in horizontal organ baths (Harvard Apparatus GmbH, March-Hugstetten, Germany). Electrical field stimulation (0.6 Hz, 2 ms, twice the diastolic threshold) was applied to the muscle [48] and the organ bath was perfused with Krebs solution (95% O_2; 5% CO_2; 30°C) at a rate of 5 ml/min. Tension was recorded using a force transducer (HSE F30; Type 372, Harvard Apparatus GmbH). Following a 60-minute stabilization period, the maximal tension (L_{max}) was found by stretching the papillary muscles with 10 µm increments and the experiments were then performed at 90% of maximal tension. After equilibration, cumulative concentration–response curves of isoprenaline (0.1 nM-10 µM) were obtained.

2.4. Chemicals

EMPA tablets (Jardiance®, 25 mg, Boehringer Ingelheim) were purchased from a local pharmacy. The ingredients of Krebs solution, STZ and isoprenaline were obtained from Sigma Aldrich (Darmstadt, Germany). Isoflurane was received from Adeka (Samsun, Turkey). A Bayer glucometer and glucose test strips (Contour Plus, Switzerland) were used to measure blood glucose level.

2.5. Data analysis

The efficacy of isoprenaline in the papillary muscle experiments was determined by fitting a 3-parameter model (top, bottom, log EC_{50}) of concentration-response curve to the experimental data from each experiment to derive an estimated maximum effect (E_{max}). Data are expressed as mean ± SD and shown as bar graphs overlaid with scatter plots for increased transparency [49]. To avoid misleading y-axis scaling, all y-axes start at 0 [50]. All statistical analyses were performed with Prism (version 9, GraphPad, La Jolla, CA, USA). As the study was of exploratory nature, the obtained data were not interpreted as hypothesis-testing but only as descriptive. We did not perform p-value calculations for the parameters intended for model validation but only for our target parameter, the Emax for isoprenaline in the isolated papillary muscle experiments. For this purpose, ANOVA was performed; as this did not yield a low p-value, no post-tests were performed for intergroup comparisons.

3. Results

3.1. General animal characteristics

STZ injection markedly increased blood glucose, which was substantially attenuated but not fully abolished by EMPA (Figure 1), thereby validating the induction of diabetes by STZ and the antidiabetic efficacy of low-dose EMPA in this model. While the control and EMPA treated control groups had similar blood glucose and body weight profiles, diabetic rats had a reduced body weight that was not affected by EMPA (Figure 1). Heart weight was similar across all groups leading to a slightly increased heart/body weight ratio in diabetic and EMPA treated diabetic rats (Figure 1).

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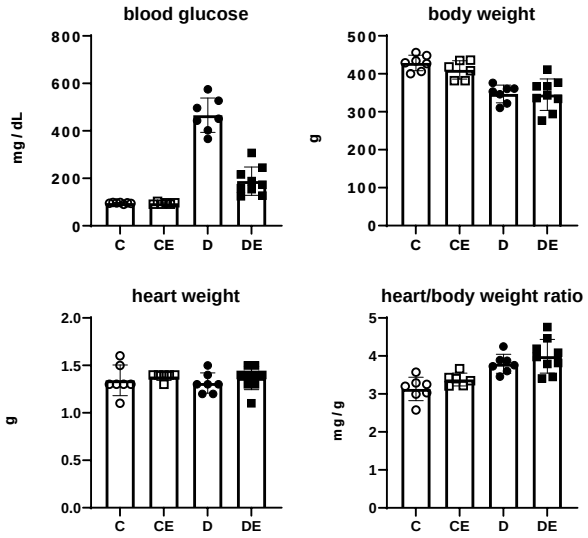

Figure 1. Blood glucose, body weight, heart weight and heart/body weight ratio in control (C; n=7), EMPA-treated control (CE; n=6), diabetic (D; n=7) and EMPA-treated diabetic rats (DE; n=9). Each data point represents one animal, bars represent means ± SD. A statistical analysis was not performed because these measurements are intended for model validation only.

3.2. In vivo baseline hemodynamic parameters

Systolic blood pressure, diastolic blood pressure and mean arterial pressure were measured when the PV loop catheter was inserted in the carotid artery. The values were slightly reduced in the diabetic groups, but no improvement was observed in EMPA treated diabetic group (Table 1). Cardiac function parameters were assessed by using the PV loop catheter and most of these parameters are summarized in Table 1.

Table 1. *In vivo* basal hemodynamic parameters in control (C; n=7), EMPA-treated control (CE; n=5), diabetic (D; n=6) and EMPA-treated diabetic rats (DE; n=8). Values are presented as means ± SD. SBP: Systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure HR: heart rate; CO: Cardiac output; SW: Stroke work; ESV: End-systolic volume; EDV: End-diastolic volume; SV: Stroke volume; EDVI: End-diastolic volume index; ESVI: End-systolic volume index; CI: cardiac index; SVI: stroke volume index. A statistical analysis was not performed because these measurements are intended for model validation only.

	C	CE	D	DE
SBP, mm Hg	133±32	123±11	114±16	116±13
DBP, mm Hg	95±25	96±16	85±7	90±10
MAP, mm Hg	120±29	114±12	105±13	107±12
HR, beats/min	285±25	282±11	250±22	242±19
CO, ml/min	55±12	58±9	39±5	49±8
SW, mm Hg/µl	22±4	19±3	16±3	19±4
EDV, µl	388±28	374±50	357±70	391±53
ESV, µl	193±24	167±26	199±45	188±29
SV, µl	195±38	207±30	157±30	203±34
EDVI, µl/g·10^3	0.91±0.09	0.90±0.15	1.03±0.20	1.14±0.19
ESVI, µl/g·10^3	0.45±0.08	0.40±0.57	0.57±0.12	0.55±0.10
CI, ml/min·g·10^3	129±27	141±23	113±15	143±26
SVI, µl/g·10^3	0.45±0.09	0.50±0.08	0.46±0.10	0.59±0.12

The parameters closely related to systolic and diastolic function are shown in Figure 2 and 3. Our results indicate that some of the hemodynamic parameters such as heart rate,

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cardiac output and stroke work were impaired in the diabetic group; however, EMPA treatment did not improve these parameters in diabetics. Other parameters were found similar in all four groups (Table 1). The systolic function parameters end-systolic pressure, rate of contraction and ejection fraction decreased in the diabetic group. These parameters were slightly greater in EMPA treated diabetics compared to diabetic group (end-systolic pressure; C, 107±6.5; CE, 105±5.8; D, 92±9.3; DE, 97±16.5; rate of contraction; C, 6790±655; CE, 6437±434; D, 4879±664; DE, 5276±674; ejection fraction (%); C, 50±7.4; CE, 55±3.1; D, 44±4.0; DE, 52±4.7) (Figure 2). On the other hand, EMPA did not affect systolic function in control animals.

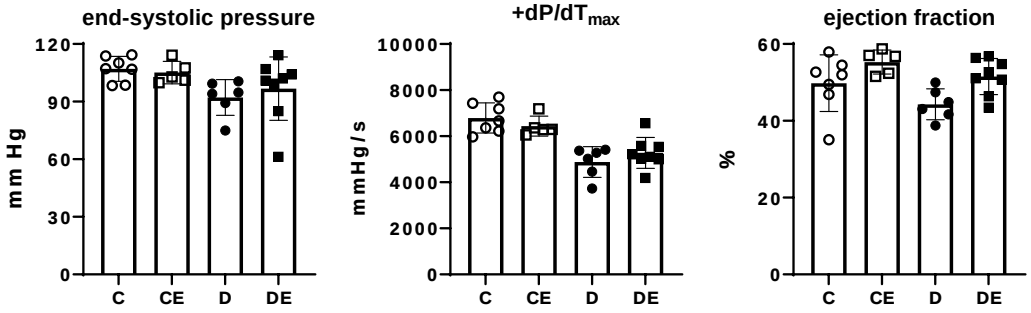

Figure 2. *In vivo* basal systolic parameters. End-systolic pressure, rate of contraction (+dp/dt) and ejection fraction in control (C; n=7), EMPA-treated control (CE; n=5), diabetic (D; n=6) and EMPA-treated diabetic rats (DE; n=8). Each data point represents one animal, bars represent means ± SD. A statistical analysis was not performed because these measurements are intended for model validation only.

Similarly, diastolic function was impaired in the diabetic group. EMPA shows a slight improvement, however, was unable to normalize the relaxation rate (rate of relaxation ; C, -5972±833.2; CE, -5413± 414.4; D, -4111±607.1; DE, -4532±999.5), end-diastolic pressure (C, 10.52±2.13; CE, 9.96±3.52; D, 8.39± 2.63; DE, 9.70±1.75) or the time constant of isovolumic relaxation (Tau) in diabetics (Tau logistic; C, 18.49±1.22; CE, 19.47±1.42; D, 22.79± 3.01; DE, 22.21±0.97) (Figure 3).

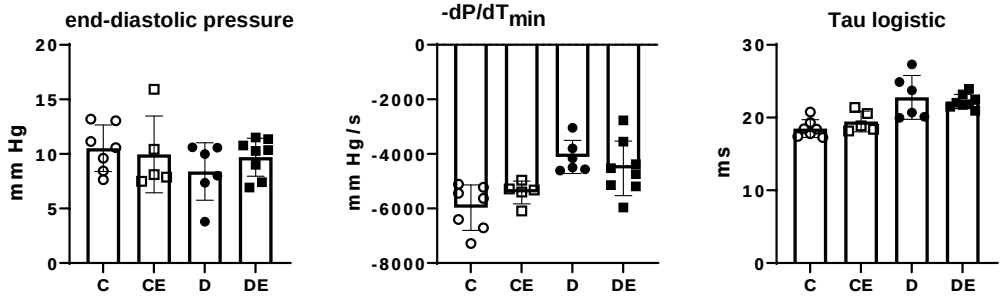

Figure 3. *In vivo* basal diastolic parameters. End-diastolic pressure, rate of relaxation (-dp/dt) and isovolumic relaxation time constant (Tau) in control (C; n=7), EMPA-treated control (CE; n=5), diabetic (D; n=6) and EMPA-treated diabetic rats (DE; n=8). Each data point represents one animal, bars represent means ± SD. A statistical analysis was not performed because these measurements are intended for model validation only.

Preload independent parameters (preload recruitable stroke work, end-systolic pressure-volume relationship, end-diastolic pressure-volume relationship) were measured by *vena cava inferior* occlusions for 5-second. The values are shown in Table 2. The expected deterioration was not observed in the diabetic group, so, it seems unlikely to comment on the effect of EMPA in diabetic animals, however, EMPA had no major effect in the control group.

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Taken together, these data demonstrate some degree of impairment of basal cardiac function in diabetes, but only modest beneficial effects of EMPA.

Table 2. Preload independent *in vivo* cardiac parameters end-systolic pressure-volume relationship (ESPVR), end-diastolic pressure-volume relationship (EDPVR) and preload recruitable stroke work (PRSW) in control (C; n=7), EMPA-treated control (CE; n=5), diabetic (D; n=5) and EMPA-treated diabetic rats (DE; n=7). Values are presented as means ± SD. A statistical analysis was not performed because these measurements are intended for model validation only.

	C	CE	D	DE
ESPVR	0.29±0.08	0.33±0.15	0.28±0.06	0.32±0.10
EDPVR	0.005±0.004	0.006±0.003	0.008±0.005	0.008±0.006
PRSW	53±8	58±11	56±11	53±6

3.3. Contractile responses to isoprenaline in papillary muscle

The responses to β-AR agonist isoprenaline (0.1 nM-10 μM) were evaluated in the papillary muscle isolated from the left ventricle. Although the inotropic response in diabetics seems to have decreased slightly compared to controls, this decrease was lower than expected (Figure 4). The contraction response in the EMPA group was very similar to the control group. On the other hand, treatment with EMPA did not ameliorate the decreased contractility due to diabetes (Figure 4). The p-value for the overall ANOVA (p=0.0913) was insufficient to reject the null hypothesis of inter-group differences. The corresponding pEC_{50} values in the four groups were also similar across groups (C, 7.26±0.29; CE 7.23±0.19; D: 7.22±0.19; DE: 7.32±0.31).

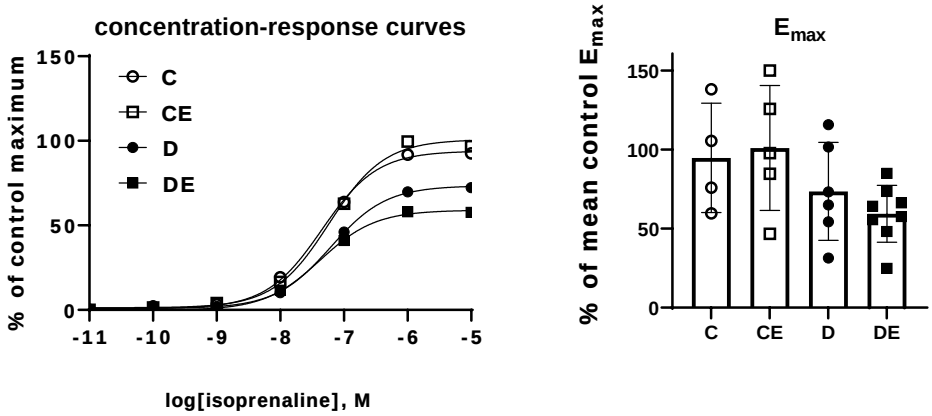

Figure 4. Concentration-response curves and maximal responses to isoprenaline in control (C; n=4), EMPA-treated control (CE; n=5), diabetic (D; n=6) and EMPA-treated diabetic rats (CE; n=8). For curves only mean values are shown for clarity, whereas each data point represents one animal and bars represent means ± SD for calculated E_{max}.

4. Discussion

SGLT2 inhibitors have shown beneficial effects in heart failure patients with and without concomitant diabetes. Desensitization of cardiac β-AR is probably the most consistent pathophysiological finding at the cellular and molecular level in heart failure and also occurs in diabetic animals, but effects of SGLT2 inhibitors on cardiac β-AR function have not yet been reported to the best of our knowledge. Therefore, a pilot study was performed to explore the effects of the SGLT2 inhibitor EMPA on responses to the β-AR agonist isoprenaline in the rat STZ model of diabetes.

4.1. Critique of methods

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SGLT2 inhibitors are approved for the treatment of T2DM and at least some (dapagliflozin and EMPA) irrespective of diabetic status also for the treatment of heart failure. According, their effects on the heart have been studied in various rodent models of T2DM [17-22,24-28]. Although SGLT2 inhibitors are not used for the treatment of T1DM, some studies have explored their cardiac effects in STZ-based rat and mouse models thereof [29-32], where they also exhibited beneficial effects. The rationale for this is based on the mechanism of action of SGLT2 inhibitors, i.e., promoting renal glucose excretion that can be useful in both T2DM and T1DM patients. Accordingly, some clinical studies have demonstrated beneficial effects of SGLT2 inhibitors in T1DM patients [51-53]. We have also used an STZ-based model. The key advantage of this is that it typically produces a more severe diabetic and cardiovascular phenotype than most models of T2DM, which facilitates detection of therapeutic effects. Previous studies in the rat STZ model have largely been based on doses of 30-60 mg/kg for rats (considerably higher doses were used in most mouse studies) [54], and studies in diabetic cardiomyopathy typically have also used similar doses [29,30,33]. Our laboratory has previously used STZ doses of 32-45 mg/kg [46,55-60]; these exhibited a phenotype with cardiac impairment including diminished β-AR mediated contractility of isolated papillary muscle [46]. We prefer these lower doses because they tend to cause less mortality than e.g., 50-60 mg/kg [61]. Therefore, the present study was based on an STZ dose of 40 mg/kg, which caused a major increase in blood glucose. Despite our hope to reduce attrition due to mortality by choosing a low STZ dose, we have observed a considerable loss of animals, which was higher than in our previous studies [46,55-60]. This led to smaller sample sizes than intended.

Our experiments have initiated EMPA treatment 13-16 weeks after STZ injection, i.e., at a time point when diabetic cardiomyopathy can be expected to have fully developed. This is later than most other studies in the field, which started SGLT2 inhibitor treatment in most cases ≤1 week after STZ injection [21,23,24,29,31-34], and only rarely at later time points such as 2 weeks [30] or 8 weeks after STZ injection [22]. We consider our approach to have higher translational validity as the diagnosis of T2DM typically is made only at a time considerably after onset of the disease. On the other hand, structural cardiac damage including fibrosis [22,30] may have fully developed at such late time points, which could make it more difficult for any medication to reverse this. In other words, most previous studies in this field have used study designs that are largely preventive based on the early start of the intervention, whereas ours and one other study [22] belong to the very few applying a therapeutic design, i.e., starting the SGLT2 inhibitor treatment after cardiomyopathy has developed.

Meta-analyses have shown that the beneficial effects of dapagliflozin and EMPA in heart failure patients are quantitatively similar [62] and major international guidelines assume this to be a class effect [15,16]. Rodent studies with EMPA have mostly used daily oral doses of 10-30 mg/kg [7], and studies related to diabetic cardiomyopathy have also used those doses [20,27,29,30,33]. While both doses appeared effective, the only direct comparative study in the field indicates that the daily 10 mg/kg dose used by us produces a less pronounced blood glucose lowering and improvement of cardiac function [33]. Our study used crushed EMPA tablets for the treatments, and the reduced blood glucose levels confirm that this resulted in an efficacious delivery of the treatment. While we cannot exclude that the excipients in the clinically used material have contributed to that, we consider this highly unlikely. In a previous study crushed dapagliflozin tablets had also effectively decreased blood glucose [63]. As we discussed also in that study, using tablets may enhance the translational value of the study as it better mimics the clinical condition. Various approaches have been used to assess cardiac function in rodent models of diabetes. While the most frequently used technique is echocardiography [20-22,24-28,31] others including PV loop analysis [18,29], cardiac catheters in general [33], ECG analysis [26], heart weight [19,23,32] and cardiac enzymes such as LDH and CK-MB have also been used [34]. Our *in vivo* studies are based on PV loop analysis, which like other approaches using

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left ventricular catheterization is a robust method to determine systolic and diastolic function.

4.2. Model validation

Our blood glucose data demonstrate the expected elevation upon treatment with STZ and lowering with EMPA (Figure 1). This was associated with reductions of end-systolic pressure and cardiac contractility and relaxation *in vivo* (Figures 2 and 3) and reduced contractile responses in the papillary muscle preparations (Figure 4). Accordingly, our study in principle yielded the expected phenotype of diabetic cardiomyopathy. However, the degree of impairment was smaller than what is normally seen by us [46,55-60] and others in the rat STZ model [30-34]. This smaller than expected severity of cardiomyopathy can potentially be at least partly explained by the unexpectedly high mortality, and may reflect that the surviving had a less severe cardiac phenotype than the overall group.

4.3. Effects of EMPA on diabetic cardiomyopathy

Our experiments found a trend for improvement upon administration of EMPA to diabetic rats for instance for ejection fraction, relaxation, cardiac output, stroke work, stroke volume or cardiac index but not for many other parameters including responses to the β-AR agonist isoprenaline. These findings were rather disappointing. Part of this could possibly be explained by lower sample sizes than planned due to an unexpectedly high attrition and to a less severe cardiac phenotype than in our previous studies using similar STZ doses [46,55-60], which in turn may at least partly reflect that animals with a more severe phenotype may preferentially have contributed to the attrition. Both factors would markedly reduce the statistical power to detect the beneficial effects of EMPA. However, there also is a possible biological reason for the weaker effects of EMPA in the present as compared to previous studies using this or other SGLT2 inhibitors in models involving an STZ injections with or without a prior/concomitant HFD [21,23,24,29-34]. As noted above, these studies started administration of the SGLT2 inhibitor mostly ≤1 week after the STZ injection, i.e., at a time point when structural damage to the heart may yet have been rudimentary or even absent. The present data do not allow to address this possibility. However, it is noteworthy that other studies from our group using STZ injection found that it prevents a diabetic phenotype in the urinary bladder when EMPA administration starts early (prevention design) [64] but not when it starts late (treatment design) [65]. Given that diabetes often is diagnosed several years after onset, our findings may be different from those of others but nonetheless more reflective of the clinical situation. This is a testable hypothesis for future studies.

5. Conclusions

In summary, the present pilot study does not provide robust evidence for a beneficial effect of EMPA in the STZ injection-based rat model of T1DM when administration of EMPA started 13-16 weeks after STZ injection, i.e., in a therapeutic setting. This contradicts other studies reporting beneficial effects of SGLT2 inhibitors including EMPA in STZ-based diabetes model with or without a prior/concomitant HFD [21,23,24,29-34]. However, those studies initiated the treatment with an SGLT2 inhibitor much earlier (mostly within ≤1 week after STZ injection), which may reflect a preventive and not a therapeutic setting. While we cannot exclude that other factors including a surprising small severity of diabetic cardiomyopathy and a surprisingly large attrition due to mortality have contributed to our negative data, we propose that future studies should explicitly compare early/preventive and late/therapeutic settings when evaluating the effects of SGLT2 inhibitors in diabetic cardiomyopathy. In this sense, our pilot study provided what a pilot study should do, it led to a novel testable hypothesis.

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Data Availability Statement: Raw data of all experiments will be made available to qualified investigators upon reasonable request.

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