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Original Article

Sitagliptin does not improve isoprenaline-induced cardiac contractility in streptozotocin-induced diabetic rats

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ABSTRACT

Background and Aims: Sitagliptin, a dipeptidyl peptidase IV (DPP-IV) inhibitor, has been shown to have beneficial effects on the diabetic heart. Beta-adrenoceptor (β-AR)-mediated responses are impaired in diabetes. Our aim was to investigate the impact of sitagliptin on the diabetic rat heart in terms of β-AR-mediated responsiveness. In addition, we examined the expression of proteins associated with diastolic dysfunction and endoplasmic reticulum (ER) stress, as well as proteins involved in the β-AR signalling pathway.

Methods: Eight-week-old Sprague-Dawley rats were divided into control, diabetic, and sitagliptin-treated (10 mg/kg/day for 4 weeks) diabetic groups. Type 1 diabetes was induced by intraperitoneal injection of streptozotocin (STZ). Throughout the treatment period, the rats received sitagliptin orally. Cardiac β-AR responsiveness was assessed using *in vitro* papillary muscle experiments with a nonselective β-AR agonist, isoprenaline, and *in vitro* Langendorff heart preparation experiments with a β_3-AR selective agonist CL 316,243. Western blot experiments were conducted to assess the protein expression of SERCA2a, GRP78, β_3-AR, eNOS, and p-eNOS.

Results: Sitagliptin did not reduce blood glucose levels or reverse weight loss in diabetic rats. However, it improved the heart weight to body weight ratio, indicating a reduction in cardiac hypertrophy. Sitagliptin did not correct the isoprenaline-induced contractile response in the diabetic group, nor did it alter the β_3-AR mediated relaxation. Sitagliptin treatment also did not improve the downregulation of SERCA2a or the upregulation of GRP78. However, it reduced the upregulation of β_3-AR. The protein expression of eNOS and the ratio of p-eNOS to eNOS were similar among the groups.

Conclusion: This study indicates that sitagliptin treatment did not improve isoprenaline-mediated contractile responses or affect β_3-AR-mediated relaxation in the diabetic heart. However, the observed increase in β_3-AR protein expression in the diabetic heart treated with sitagliptin indicated a potential differential effect of the drug on this pathway compared to the β_1-AR signalling pathway. Further studies are needed to elucidate the precise mechanisms by which sitagliptin influences β_3-AR-mediated pathways.

Keywords: Beta adrenoceptor, Diabetes, Heart, Isoprenaline, Sitagliptin

INTRODUCTION

Dipeptidyl peptidase IV (DPP-IV) inhibitors represent a recent class of antidiabetic medications that enhance glycemic control in patients with type 2 diabetes. These inhibitors work by boosting insulin secretion from islet β-cells through increased availability of incretin hormones, thereby leading to decreased blood glucose levels (Gopal, Chahade, Kim, & Ussher, 2020). Studies in preclinical settings have demonstrated the positive impact of DPP-IV inhibitors on cardiac function (Zhou et al., 2018; Khodeer, Bilasy, Farag, Mehana, & Elbaz, 2019). However, clinical trials assessing cardiovascular outcomes have not yet shown this class to be superior to placebo in patients with type 2 diabetes and high cardiovascular risk (Scheen, 2018).

Sitagliptin, the first approved DPP-IV inhibitor, is a potent

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and highly selective member of this group (Lyseng-Williamson, 2007). Similar to other DPP-IV inhibitors, sitagliptin prevents the inactivation of incretin, thus promoting glucose-dependent insulin secretion. Experimental studies have indicated several cardiac benefits of sitagliptin. For instance, in Zucker Diabetic Fatty (ZDF) rats, 12 weeks of sitagliptin treatment improved ejection fraction and fractional shortening (Zhou et al. , 2018). Similarly, in Goto-Kakizaki rats, a model of type 2 diabetes, 20 weeks of sitagliptin treatment enhanced diastolic function (Ramírez et al., 2018). Furthermore, in streptozotocin (STZ) diabetic rats, 12 weeks of sitagliptin treatment ameliorated cardiac contraction and relaxation (Wu, Xu, Zhang, & Bao, 2019). The favourable effects of sitagliptin extend beyond the diabetic heart. In Dahl salt-sensitive rats fed a high-salt diet, 8 weeks of sitagliptin treatment improved diastolic dysfunction (Esposito et al., 2017). Sitagliptin also protects against isoprenaline-induced myocardial damage in rats (Ibrahim, Geddawy, & Abdel-Wahab, 2018). These findings show that sitagliptin may affect the heart through mechanisms beyond glucose regulation.

A notable characteristic of the diabetic heart is the diminished responsiveness of beta-adrenoceptors (β-AR) which is primarily associated with reduced β-AR expression (Dincer et al., 2001; Haley, Thackeray, Kolajova, Thorn, & DaSilva, 2015; Jiang et al., 2015). Changes in β-AR-mediated responses are critical because cardiac contractility is mainly regulated by β_1-ARs and to some extent by β_2-ARs. A third subtype, β_3-AR, is also noteworthy because it mediates a negative inotropic effect in the heart (Gauthier, Tavernier, Charpentier, Langin, & Le Marec, 1996). The role of this subtype is significant in conditions associated with catecholamine overstimulation, such as heart failure or diabetes (Moniotte & Balligand, 2003; Rozec, Noireaud, Trochu, & Gauthier, 2003).

The impact of sitagliptin on cardiac β-adrenergic responsiveness has not yet been investigated. Therefore, the present study aimed to determine whether sitagliptin treatment has a beneficial effect on cardiac β-adrenergic responsiveness independent of its metabolic benefits in the heart of STZ-induced diabetic rats.

MATERIALS AND METHODS

Animals

The study was approved by the local ethical committee of Ankara University (2014-24-161). Animal experiments were performed in accordance with the NIH Guidelines for Care and Use of Laboratory Animals. Eight-week-old male Sprague–Dawley rats (200-250 g) were purchased from Bilkent University and Gazi University. The rats were housed in the animal facility of the Faculty of Pharmacy, Ankara University, under a 12-h light/12-h dark cycle with free access to standard chow and water.

Induction of diabetes and sitagliptin treatment

Rats were randomly assigned to three groups: control (C, n=15), diabetic (D, n=20), and sitagliptin-treated diabetic (S, n=16). Diabetes was induced by intraperitoneal injection of STZ at doses of 35 or 40 mg/kg. After 72 h, glucose levels were measured using a glucose meter (VivaChek, Biotech, China) with blood samples taken from the tail. Rats with blood glucose levels (non-fasting) below 300 mg/dl received a second or third dose of STZ (40 or 45 mg/kg, respectively). In the preliminary study to determine the appropriate sitagliptin dose, STZ-diabetic rats were orally administered 10 and 30 mg/kg/day for 4 weeks, following protocols from previous studies. However, no significant decrease in blood glucose was observed at either dose (C, n = 6; D, n = 4; S (10 mg/kg), n = 5; S (30 mg/kg), n=5). Previous research suggests that a dose of 5-10 mg/kg/day is sufficient to determine the effect of sitagliptin on the heart independent of its impact on blood glucose (Reimer et al., 2012; T.-M. Lee, Chen, & Chang, 2016). To evaluate the potential cardiac effects of sitagliptin treatment independent of its metabolic effects, we administered a dose of 10 mg/kg to rats based on the literature review. Sitagliptin treatment was initiated after 10 weeks of diabetes (10 mg/kg/day, once daily, 4 weeks) (Figure 1). For this purpose, Januvia® tablets (128.5 mg sitagliptin phosphate monohydrate equivalent to 100 mg sitagliptin) were used to prepare the suspension administered orally. Distilled water was given to the C and D groups. On the day of the experiment, just before euthanasia, body weight and blood glucose levels were recorded.

Papillary muscle experiments

Rats were euthanized under aether anaesthesia and their hearts were rapidly excised. The left ventricular papillary muscle was dissected and mounted in a superfusion organ bath. The muscle was paced at a cycle length of 1700 ms with a 2-ms stimulus pulse at double threshold voltage. The muscle was perfused with Tyrode's solution containing 116 mM NaCl; 5 mM KCl; 2.7 mM CaCl2; 1.1 mM $MgCl_2$; 0.33 mM $NaHPO_4$; 24 mM $NaHCO_3$, and 5 mM glucose, flowing at a rate of 5 ml/min (30°C, 95% O2/ % CO_2, pH= 7.4). Muscle tension was recorded using a mechanoelectrical force transducer (Commat Pharmacology & Physiology Instruments, Ankara, Turkiye). The papillary muscle was allowed to stabilize for 60 min before being progressively stretched. Dose-response curves were generated at 90% of the maximum tension. The response mediated by β_1- and β_2-ARs was assessed using isoprenaline, a non-selective β-AR agonist, across a range of concentrations from 0.1 nM to 30 mM.

Figure 1. Design of the study and experimental timeline.

Langendorff-perfused cardiac experiments

Rats were euthanized under ether anaesthesia. Hearts were rapidly excised in ice-cold Krebs-Henseleit solution (in mmol/L; 120 NaCl, 4.8 KCl, 1.25 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 11 glucose; pH 7.4) and then retrogradely perfused at a rate of 10 ml/min (37°C, 95% O_2/ 5% CO_2). The heart rate was maintained at 300 bpm by pacing the right ventricle with a stimulator (Grass Instrument Inc., Quincy, MA, USA). A latex balloon connected to a pressure transducer was inserted into the left ventricle, and the volume of the balloon was adjusted to set the left ventricular end-diastolic pressure to 10 mmHg at the beginning of each experiment. Hearts were perfused with a constant flow for 30 min until they reached a steady state. The β_3-AR-mediated relaxation response was then assessed using the selective agonist CL 316,243, which was added to the Krebs-Henseleit solution at increasing concentrations (0.1 pM-1 µM). Left ventricular developed pressure (LVDP, the difference between LV systolic pressure and end-diastolic pressure) was measured using software (version 3.5.0 for Windows, Biopac Systems Inc., Santa Barbara, CA). Data were recorded online via an analogue-to-digital interface (model MP100; Biopac Systems Inc.).

Western Blotting

The left ventricular tissue was homogenised using RIPA solution (comprising RIPA buffer, sodium orthovanadate and protease inhibitor cocktail). After sonication, the homogenate was agitated for 2 h at +4°C and then centrifuged at 16.000g for 30 min at +4°C. Protein concentration was determined by the bicinchoninic acid (BCA) assay. Protein samples ranging from 10-100 µg were loaded onto a polyacrylamide gel (TGX Fast Cast, 7.5%) and subsequently transferred to a polyvinylidene difluoride (PVDF) membrane at 100 V for 2 or 4 h. The membranes were blocked with 5% bovine serum albumin (BSA) in tris-buffered saline containing 0.1% Tween 20 (TBST), followed by overnight incubation with primary antibodies at +4°C. The primary antibodies used included β_3-AR (1/500), endothelial nitric oxide synthase (eNOS) (1/500), phosphorylated eNOS (p-eNOS) (1/1000), sarcoplasmic reticulum calcium ATPase 2a (SERCA2a) (1/2000), and glucose-regulated protein 78 (GRP78) (1/1000). After washing, the membranes were incubated with secondary antibodies for 2 h at +4°C, including anti-chicken (1/3000) and anti-rabbit (1/2000) antibodies. Protein bands were visualised using enhanced chemiluminescence assay and exposed to film. Quantification of protein bands was performed using ImageJ software (NIH, USA), and expression levels were normalised to the housekeeping gene, α-tubulin (1/10.000).

Statistical Analysis

Results are presented as mean ± standard deviation (SD). Statistical significance was assessed using one-way ANOVA, followed by Bonferroni's multiple comparison test to evaluate differences between groups. A p-value < 0.05 was considered statistically significant. All statistical analyses and graph plotting were performed using Prism software (version 9.5.0 GraphPad, La Jolla, CA, USA).

Chemicals

Sitagliptin (Januvia, 100 mg, Merck Sharp & Dohme, Levent, Istanbul), streptozotocin (Sigma-Aldrich, Missouri, USA), isoprenaline (Sigma-Aldrich, Missouri, USA), CL 316,243 (Sigma-Aldrich, Missouri, USA), anti-β_3 antibody (ab59685, Abcam, Cambridge, UK), anti-eNOS antibody (9572S, Cell Signaling Technology, Massachusetts, USA), anti-p-eNOS (ser1177) antibody (9571S, Cell Signaling Technology, Massachusetts, USA), anti-SERCA2a (4388, Cell Signaling Technology, Massachusetts, USA), anti-GRP78 (ab21685, Abcam, Cambridge, UK) anti-α-tubulin antibody (ab4074, Abcam, Cambridge, UK), anti-chicken antibody (29710, AnaSpec, Fremont, USA), anti-rabbit antibody (7074S, Cell Signaling Technology, Massachusetts, USA), RIPA buffer (Sigma-Aldrich, Missouri, USA), BCA kit (Thermo Fischer, Massachusetts, USA), TGX Fast Cast (Bio-Rad, California, USA), ECL kit (Thermo Fischer, Massachusetts, USA), BSA (A7030, Sigma-Aldrich, Missouri, USA), Film (Kodak, New York, USA).

RESULTS

General characteristics of rats

Blood glucose levels were significantly higher in group D after 14 weeks of diabetes; however, sitagliptin treatment did not reduce the blood glucose levels (Table 1). Diabetic rats lost body weight as expected, with body weight remaining lower in the S group (Table 1). Heart weight was comparable between

the groups (Table 1). The ratio of heart weight to body weight was higher in the D group and was significantly improved by sitagliptin treatment (Table 1).

β_1- and β_2-AR-mediated contractile responses in papillary muscles

The dose-dependent contractile response to isoprenaline in the papillary muscle was attenuated in group D and was not recovered by the treatment (Figure 2A). Similarly, the maximal response to the agonist was reduced in group D and did not increase with sitagliptin treatment (E_{max}; C: 153.50±61.19; D: 46.18±24.07; S: 71.92±32.51; %, Figure 2B).

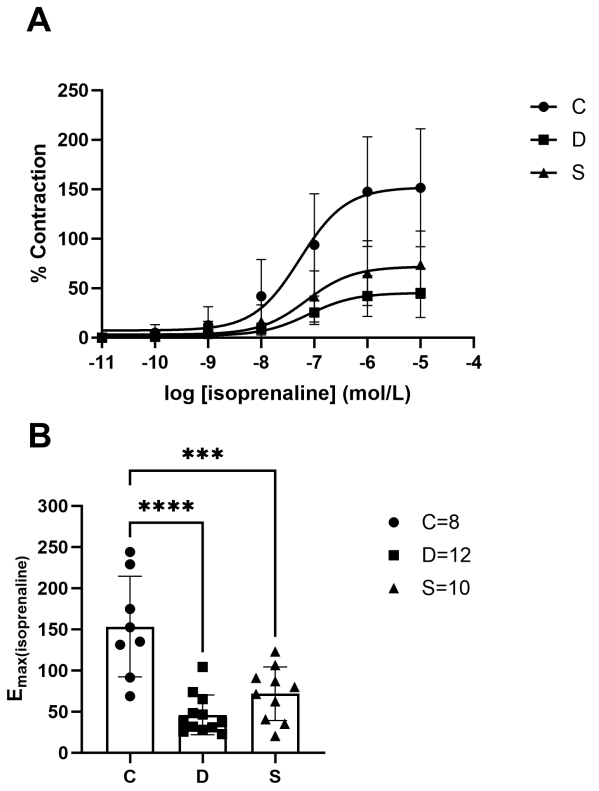

Figure 2. Isoprenaline-induced contractility. **A.** Cumulative concentration response curve. **B.** Maximum response. C, control group; D, diabetic group; S, sitagliptin-treated diabetic group. E_{max}: maximum effect. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's multiple comparison test. ***, p<0.001, ****, p<0.0001.

β_3-AR-mediated relaxation response in Langendorff heart preparation

β_3-AR-mediated relaxation was determined using the selective agonist CL 316,243, which induced dose-dependent relaxation in Langendorff-perfused rat hearts. The negative inotropic effect was enhanced in group D; however, sitagliptin treatment did not reduce it (Figure 3A). The maximal response was also higher in the D and S groups (E_{max}; C: 91.34±7.66; D: 80.15±11.71; S: 87.37±6.97; %, Figure 3B).

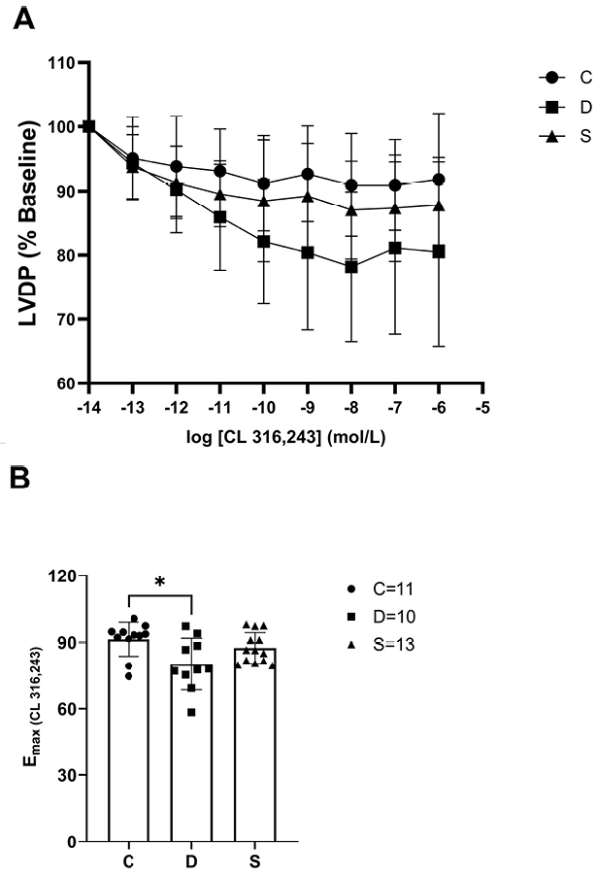

Figure 3. CL 316,243-mediated relaxation. **A.** Cumulative concentration response curve. B. Maximum response. C, control group; D, diabetic group; S, sitagliptin-treated diabetic group. E_{max}: maximum effect. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's multiple comparison test. *, p<0.05.

Protein expression of SERCA2a and GRP78

SERCA2a was downregulated in the D group but not improved in the S group (C: 100.00±14.20 D: 58.59±25.20; S: 60.24±31.78, Figure 4A). The protein expression of GRP78, a marker of ER stress, was slightly increased in the D group but not significantly altered in the S group (C: 100.00±19.32; D: 140.00±50.42; S: 128.90±22.50, Figure 4B).

Protein expression of β_3-AR, eNOS, and p-eNOS

β_3-AR was upregulated in group D and was significantly decreased by sitagliptin treatment (C: 100.00±37.90; D: 279.60±22.32; S: 180.20±32.16, Figure 5A). eNOS expression, on the other hand, did not differ between groups (eNOS; C:100.00±22.10; D: 89.56±36.83; S: 91.99±38.42; Figure 5B). p-eNOS or the ratio of p-eNOS to eNOS also did not change

Table 1. General characteristics of the rats.

	C (n=14)	**D (n=20)**	**S (n=16)**
BG (mg/dl)	99.93±9.84	502.90±89.38****	459.20±103.40****
	C (n=15)	**D (n=16)**	**S (n=16)**
BW (g/g)	394.70±46.29	295.10±54.88****	318.80±37.49***
HW (g)	1.55±0.33	1.44±0.24	1.35±0.22
HW/BW (g/g)	0.00393±0.00048	0.00496±0.00062****	0.00424±0.00058##

BG, blood glucose; BW, body weight; HW, heart weight. ***, p<0.001, ****, p< 0.0001 compared to C; ##, p<0.01, compared to D. C, control group; D, diabetic group; S, sitagliptin treated diabetic group

significantly despite a slight increase in the D and S groups (p-eNOS; C: 100.00±32.65; D: 154.10±82.70; S: 86.33±28.74; p-eNOS/eNOS; C: 100.00±17.38; D: 174.70±123.50; S: 107.40±59.00; Figure 5C and Figure 5D).

DISCUSSION

The present study indicates that sitagliptin treatment did not improve isoprenaline-stimulated β-AR-mediated cardiac contractility in STZ-induced diabetic rats. In addition, it did not reduce hyperglycemia or prevent body weight loss in the diabetic group. However, the study identified one positive outcome of sitagliptin, which is an improvement in cardiac hypertrophy in diabetes, as evidenced by the heart weight/body weight ratio.

β-ARs play a critical role in cardiac contractile response (Brodde, Michel, & Zerkowski, 1995), and the impact of diabetes on β-AR-mediated contractility has been extensively studied. Despite conflicting results in the literature, reduced β-adrenergic responsiveness is often associated with diabetic heart (Erdogan, Michel, & Arioglu-Inan, 2020). Consistent with these observations, our study revealed attenuated isoprenaline-induced contraction in the papillary muscles of diabetic rats, a response not improved by sitagliptin treatment. This lack of improvement may be linked the persistent hyperglycemia in diabetic rats, as sitagliptin failed to correct this metabolic imbalance. However, this explanation appears unlikely given that studies in non-diabetic models have demonstrated beneficial cardiac effects of sitagliptin (Esposito et al., 2017; Ibrahim et al., 2018). Our findings on blood glucose are unsurprising, as the antihyperglycemic effect of this drug class is primarily driven by insulin secretion stimulation, which is compromised in the STZ diabetic rat model due to pancreatic β-cell destruction. Nevertheless, varying results regarding glycemic control with DPP-IV inhibitors in this model have been reported (Marques et al., 2019; Kizilay, Ersoy, Cerkezkayabekir, & Topcu-Tarladacalisir, 2021).

The beneficial cardiovascular effects of DPP-IV inhibitors have been demonstrated in numerous preclinical studies, including those involving cardiac pathologies unrelated to diabetes (Nakajima et al., 2019; Yamaguchi et al., 2019). Among these investigations, Connelly et al. reported that high-dose sitagliptin treatment did not reduce blood glucose; however, it did improve certain cardiac parameters and adverse remodelling induced by myocardial infarction in STZ-diabetic rats (Connelly et al., 2013). In addition, sitagliptin treatment was found to reduce passive left ventricular stiffness in obese type 2 diabetic mice (Hamdani et al., 2014). In this study, an increase in left ventricular stroke volume was observed, which was attributed to the stimulation of the cardiac cyclic guanosine monophosphate (cGMP)/cGMP-dependent protein kinase (PKG) pathway, rather than glycemic control. In contrast to these reported findings, our study did not observe any beneficial effect of sitagliptin on cardiac contractility.

It has been suggested that cardiac contraction or relaxation in response to β-adrenergic agonists may be influenced by altered SERCA2a activity (Kranias & Hajjar, 2012). Furthermore, the SERCA2a regulator phospholamban (PLN) has been implicated as a key component of the β-adrenergic agonist-induced cardiac response (Kranias & Hajjar, 2012), as demonstrated in PLN-deficient mice (Wolska, Stojanovic, Luo, Kranias, & Solaro, 1996). In the current study, we observed the downregulation of SERCA2a in the diabetic heart, a common occurrence in the STZ-diabetic rat model (Arioglu-Inan, Ozakca, Kayki-Mutlu, Sepici-Dincel, & Altan, 2013). Additionally, GPR78, an ER stress marker, was found to be upregulated in the diabetic heart. The reduced expression of SERCA2a may be associated with increased ER stress, similar to the findings in OLETF diabetic rats (Takada et al., 2012). In our study, sitagliptin could not correct either the downregulation of SERCA2a or the upregulation of GPR78. Thus, decreased SERCA2a expression could have contributed to the impaired contractile response observed in the diabetic heart. Our findings indicating that sitagliptin did not improve β-AR-mediated contractility in diabetic rats may

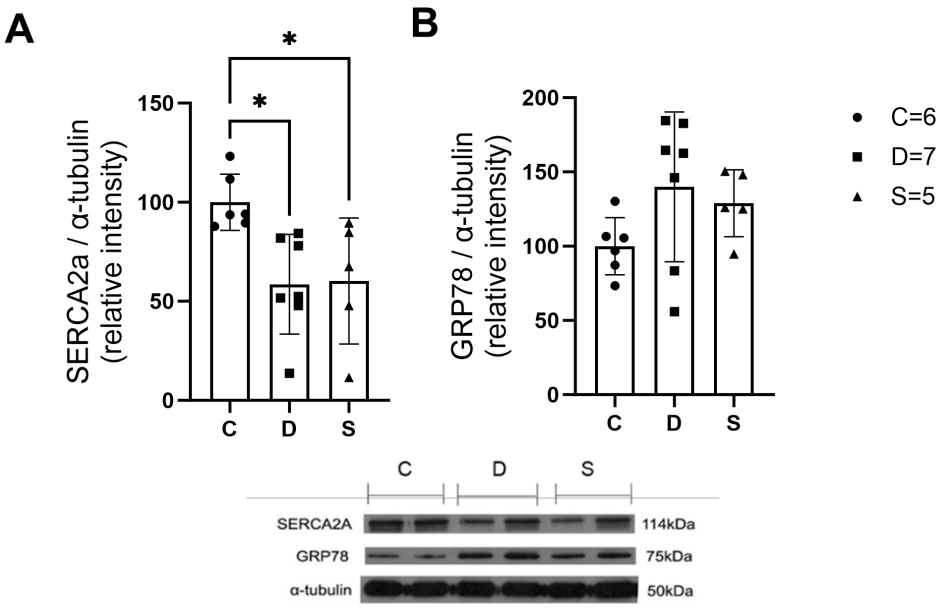

Figure 4. Protein expression levels of SERCA2a and GRP78. **A.** % relative intensity of SERCA2a. **B.** % relative intensity of GRP78. C, control group; D, diabetic group; S, sitagliptin-treated diabetic group. SERCA2a, sarcoplasmic reticulum calcium ATPase 2a; GRP78, glucose-regulated protein 78. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's multiple comparison test. *, $p<0.05$.

also be attributed to the persistent downregulation of SERCA2a, as the expression of this channel remained low in the treated group compared with the control. Nevertheless, we acknowledge that the expression of SERCA2a may not be the sole determinant of heart contractility.

Our findings regarding SERCA2a or GPR78 are not consistent with the current literature, as most studies have reported positive effects of DPP-IV inhibitors on the expression of SERCA2a and ER stress markers. For instance, sitagliptin treatment improved the downregulation of SERCA2a in ventricular myocyte from spontaneously hypertensive rats (T. Lee et al., 2013). Similarly, Aroor et al. (2013) demonstrated that linagliptin, another DPP-IV inhibitor, increased SERCA2a expression in Zucker-obese rats. In addition, sitagliptin reversed the upregulation of GRP78 and C/EBP homologous protein (CHOP) in the aorta of rats fed a high-fat diet (Cao et al., 2021) . It also reduced the upregulation of GRP78 in the testes of STZ-diabetic rats (Kizilay et al. , 2021). Moreover, sitagliptin treatment corrected the increased mRNA expression of CHOP, another ER stress marker, in the livers of insulin-resistant rats (Ahmed, Ali, Mohamed, Rashed, & Mohamed, 2021).

In our study, we also investigated the β_3-AR-mediated cardiac response. Using the Langendorff heart preparation, we observed that the β_3-AR-mediated negative inotropic effect was augmented in the diabetic heart, consistent with our previous findings (Kayki-Mutlu, Arioglu-Inan, Ozakca, Ozcelikay, & Altan, 2014). Furthermore, we noted an upregulation of β_3-ARs, which is in line with our previous studies of this subtype being elevated in STZ-diabetic rats (Dincer et al. , 2001; Amour et al., 2007). Sitagliptin treatment had no effect on the augmented relaxation response; however, it markedly reduced the upregulation of β_3-ARs. Given that the signalling pathway of cardiac β_3-ARs is proposed to involve nitric oxide (NO)(Gauthier et al., 1998), we examined the expression of eNOS, the enzyme responsible for NO production in cardiac tissue. The expression of eNOS was comparable across all the groups. We also measured the expression of phosphorylated eNOS (p-eNOS), as it has been suggested that diabetes may alter the phosphorylation of this enzyme without affecting the total protein levels (Kayki-Mutlu et al. , 2014). However, the expression of p-eNOS and the ratio of p-eNOS to eNOS were comparable in all groups, aligning with our previous results (Arioglu-Inan et al. , 2013). In support of our results, Hamdani et al. (2014) also reported unaltered eNOS phosphorylation in sitagliptin-treated obese type 2 diabetic mouse hearts. In contrast, Aroor et al. reported that both total and p-eNOS were upregulated in Zucker-obese rats (Aroor et al., 2013). Therefore, further studies are required to investigate the impact of DPP-IV inhibitors on eNOS/p-eNOS expression.

CONCLUSION

Contrary to numerous studies reporting the beneficial cardiac effects of DPP-IV inhibitors, our study found that sitagliptin treatment did not improve β-AR-mediated contractility in STZ-diabetic rat hearts. The lack of metabolic control in our study does not appear to account for this discrepancy, given that cardiac benefits of this drug class have also been reported in non-diabetic models. However, our current dataset is insufficient to

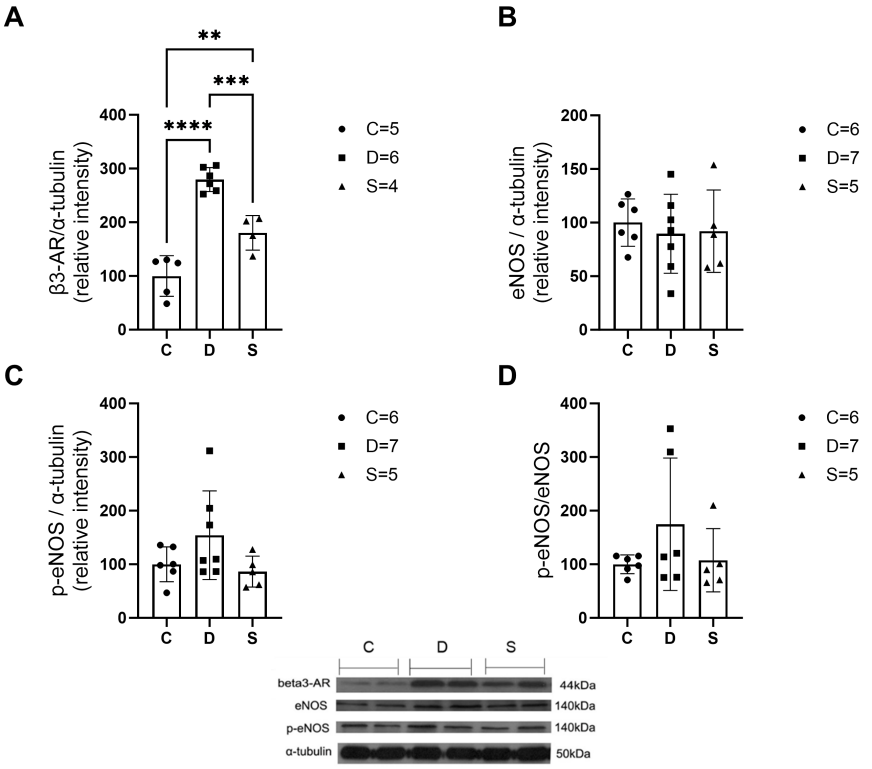

Figure 5. Protein expression levels of β_3-AR, eNOS, p-eNOS, and p-eNOS/eNOS. **A.** % relative intensity of β_3-AR. **B.** % relative intensity of eNOS. C. % relative intensity of p-eNOS. **D.** the ratio of p-eNOS to eNOS. C, control group; D, diabetic group; S, sitagliptin-treated diabetic group. β_3-AR, beta-3 adrenoceptor; eNOS, endothelial nitric oxide synthase; p-eNOS, phosphorylated eNOS. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's multiple comparison test. **, p<0.01, ***, p<0.001, ****, p<0.0001.

fully elucidate the differences between our findings and those of other research groups. Therefore, future studies are needed to clarify the effects of sitagliptin and other DPP-IV inhibitors on β-AR mediated cardiac responses.

STUDY LIMITATIONS:

This study has several limitations. First, a sitagliptin-treated control group was not included, which precluded us from assessing the potential effects of sitagliptin on the heart of healthy rats. Second, we focused solely on the expression of SERCA2a but not PLN. Exploring alterations in PLN or its phosphorylation could have provided insights into the improved contractile response despite decreased SERCA2a expression. Third, our Western blot experiments did not yield data on β_1-AR protein expression. As a result, we lack information on the components of the β_1- or β_3-AR-mediated signalling pathways in the heart, preventing us from commenting on either β_1-AR-mediated contractility or β_3-AR-mediated relaxation.

Ethics Committee Approval: The study was approved by the local ethical committee of Ankara University (2014-24-161).

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study: C.U.B., E.A.İ., V.M.A.; Data Acquisition: C.U.B., B.R.E., A.E.M., G.K.M., Z.E.Y.D., İ.K., V.M.A., E.A.İ.; Data Analysis/Interpretation: C.U.B., B.R.E., E.A.İ.; Drafting Manuscript: C.U.B., B.R.E., G.KM., E.A.İ., V.M.A.; Critical Revision of Manuscript: C.U.B., B.R.E., A.E.M., G.K.M., Z.E.Y.D., İ.K., V.M.A., E.A.İ.; Final Approval and Accountability: C.U.B., B.R.E., A.E.M., G.K.M., Z.E.Y.D., İ.K., V.M.A., E.A.İ.

Conflict of Interest: The authors have no conflict of interest to declare.

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