Review
Expression and Signaling of β-Adrenoceptor Subtypes in the Diabetic Heart

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Abstract: Diabetes is a chronic, endocrine disorder that effects millions of people worldwide. Cardiovascular complications are the major cause of diabetes-related morbidity and mortality. Cardiac β₁- and β₂-adrenoceptor (AR) stimulation mediates positive inotropy and chronotropy, whereas β₂-AR mediates negative inotropic effect. Changes in β₁-AR responsiveness are thought to be an important factor that contributes to the diabetic cardiac dysfunction. Diabetes related changes in β₁-AR expression, signaling, and β₁-AR mediated cardiac function have been studied by several investigators for many years. In the present review, we have screened PubMed database to obtain relevant articles on this topic. Our search has ended up with wide range of different findings about the effect of diabetes on β₁-AR mediated changes both in molecular and functional level. Considering these inconsistent findings, the effect of diabetes on cardiac β₁-AR still remains to be clarified.

Keywords: diabetes; heart; beta adrenoceptor

1. Introduction

Diabetes is an endocrine disorder due to partial or complete insulin deficiency or to insulin resistance in the target tissues. According to the World Health Organization (WHO) the number of diabetic individuals rose from 108 million in 1980 to 422 million in 2014 [1]. The number of people with diabetes is estimated to rise to 629 million (at ages 20–79) by 2045 [2].

Impaired glucose uptake to tissues in the absence of an insulin effect results in hyperglycemia. Despite the different characteristics and treatment strategies, both type 1 (T1DM) and type 2 diabetes (T2DM) have a similarly serious impact on the whole body because of hyperglycemia. Uncontrolled diabetes causes partly irreversible changes to various organs, which in turn lead to diabetic complications. In fact, people with T2DM are at higher risk for diabetic complications, since the diagnosis is often made when the complications already occurred [1].

Cardiovascular complications of diabetes are one of the important causes of diabetic morbidity and mortality. Diabetes is an independent risk factor for both ischemic and hemorrhagic stroke [3]. The risk of having myocardial infarction (MI) among diabetic patients without previous MI history has been found as high as nondiabetics with a previous MI history [4]. Furthermore, the prevalence of heart failure has been reported as four time higher among diabetic patients as compared to general population [5]. Although hyperglycemia is an important contributor to diabetic cardiovascular complications, regulating blood glucose is not enough to prevent them. Some clinical trials [6,7,8] have shown that major cardiovascular events were not fully prevented despite of a tight glycemic control.
β-adrenecptors (β-AR) include the β₁-, β₂- and β₃-AR subtypes [9]. β₁- and β₂-AR have been considered as the only subtypes until the third one was cloned in 1989 by Emorine et al. [10]. The β₃-AR was first detected in rodent the adipose tissue where it mediates lipolysis and thermogenesis [11]. In 1996, Gauthier et al. [12] reported the presence of this subtype in human endomyocardial biopsies. Despite the clinical trials on β₃-AR as a therapeutic target in heart failure, there is an ongoing debate on their presence in the healthy human heart [13].

Cardiac β₁- and β₂-AR essentially contribute to the control of inotropy and chronotropy [14,15,16]. Both subtypes are coupled to a stimulatory G protein (Gs) [17] and their signaling pathways include stimulation of adenylyl cyclase (AC), cyclic AMP (cAMP) formation and protein kinase A (PKA) activation. Following activation of AC and cAMP production, PKA is activated and phosphorylates L type Ca²⁺ channels [18]. Increased Ca²⁺ influx stimulates Ca²⁺ release from the sarcoplasmic reticulum (SR) and cytosolic Ca²⁺ levels are elevated. This enables cardiac contraction. β₂-AR, on the other hand, have a dual coupling. This subtype has been suggested to couple also to an inhibitory G protein (Gi) in rat cardiomyocytes since pretreatment with pertussis toxin (PTX) resulted in an enhanced positive inotropic response [19]. Similarly, the β₂-AR agonist-mediated contractile response or [Ca²⁺]: transient amplitude were increased when murine cardiomyocytes were treated with PTX [20]. Furthermore, β₂-AR mediated inotropic effect through Gi coupling also involves the cAMP-PKA signaling pathway as the response was abolished in the presence of a cAMP inhibitor [20]. It has been recently reported that the β₂-AR-G protein interaction (coupling either to Gi or Gs) is regulated by local membrane charge [21].

The β₃-AR has some structural differences as compared to β₁- and β₂-AR such as being less sensitive to agonist stimulated desensitization because of lack of a phosphorylation site for PKA and β-AR kinases [22,23,24]. The β₃-AR gene of rats or mice has 79% homology with the human ortholog [25]. Hence, an interspecies difference of the expression and function of β₃-AR [26] should be also considered. β₃-AR are coupled to Gs and Gi, and the latter mediates a negative inotropic effect in the heart through a signaling pathway including nitric oxide synthase (NOS)-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) [27,28]. The expression of β₃-AR in the healthy human heart is limited [13]. Of note, β₃-AR have been found upregulated in some cardiac pathologies such as heart failure [29] and other hypoxic conditions [30]. This effect has been suggested as a preventive mechanism as the heart is exposed to overstimulation by catecholamines in these pathologies [31,32]. This idea inspired clinical trials that investigate the effectiveness of a β₃-AR agonist mirabegron on heart disease [33,34].

Diabetes has been shown to affect both the expression of β-AR subtypes and β-AR mediated responsiveness [35]. The changes in the expression of β-AR subtypes or related signaling pathways significantly contribute to cardiac dysfunction in this pathology. Thus, in the current review we aimed to discuss the expression and signaling pathways of β-AR in the diabetic heart. For this purpose, we have used ‘diabetes, heart, beta adrenerceptor’ and ‘diabetes, heart, beta adrenergic receptor’ keywords combination to search relevant articles in PubMed database.

2. β-Adrenoceptors in the Diabetic Heart

Diabetes causes impaired cardiac function in which decreased β-AR mediated responsiveness has a major role [35]. This may at least partly be due to alterations in the expression of the β-AR subtypes and the signaling pathways they couple to. These effects of diabetes may differ between the β-AR subtypes. The contractile response mediated by β₁- and β₂-AR after stimulation with isoprenaline was reduced in streptozotocin (STZ)-induced diabetic rat papillary muscle [36,37,38,39]. On the other hand, β₂-AR mediated relaxation was increased in the Langendorff perfused heart of STZ diabetic rats [40,41]. Similarly, diabetes has resulted in decreased expression of β₁- and β₂-AR whereas β₃-AR have been found to be upregulated in this pathology [41,42,43].

Hereafter, we divide each of the three subsections mainly by type of diabetes and secondarily by species. As STZ injections are by far the most frequently applied model of TIDM, subsequent data on TIDM models always refer to that model unless explicitly noted otherwise.
2.1. mRNA and Protein Expression

Both mRNA and protein expression of β-AR subtypes in diabetes have been investigated, mostly in animals but to a more limited extent also in humans. Both T1DM and T2DM animal models have been used. Fewer experimental studies have been done in models of T2DM as compared to T1DM. This may have resulted from the fact that using T1DM models, particularly STZ injections is easier and requires less resources. Various species such as rodents, swine, dog, or hamster have been used. The duration of diabetes in such reports varied widely from 4 days to 13 months.

The β-AR expression at the protein level has been studied mostly by using radioligand binding assays and immunoblot studies. β-AR mRNA expression has been determined by using PCR. In radioligand binding studies, [3H]-CGP 12,177, [3H]-dihydroalprenolol ([3H]-DHA), [125I]-iodocyanopindolol and [125I]-iodopindolol have been used as ligands for detection of β-AR. In most of these studies, except one [44], changes in β-AR protein density level have been given without any subtype distinction. However, in the concentration of the ligands used in these studies, they do not detect β3-AR [45]. Thus, radioligand binding studies effectively provide information on the regulation of β1- and β2-AR, but not of β3-AR. Unlike radioligand binding studies, immunoblotting is used to understand subtype selective regulation of β-AR. However, most of the commercial β-AR subtype antibodies are reported to have poor target selectivity [46]; accordingly reported findings may not be true reflections of the expression of β3-AR protein. These are important points that should be considered when interpreting the studies. In this section, firstly, changes in protein level (data obtained from radioligand binding and immunoblotting studies) and then changes in mRNA level are described separately in both T1DM and T2DM.

2.1.1. mRNA and Protein Expression in Type 1 Diabetes Mellitus

The expression of β-AR in the diabetic rat heart has been first reported by Savarese and Berkowitz in 1979 [47]. They have demonstrated that the number of β-AR were 28% decreased in ventricular tissue from diabetic Sprague Dawley rats as assessed by [3H]-DHA binding 8 weeks after STZ injection. Such, downregulation of β-AR has been confirmed in many studies by using different methods at both protein and mRNA level (Table 1). As mentioned in the previous section, the decrease of the expression of β-AR subtypes has not been classified separately in most studies that performed radioligand binding assay to determine changes in receptor protein level [48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71]. However, unchanged β-AR density has been reported in female Wistar rats after 8-days of diabetes [72] and in 14-day diabetic male Wistar rats [73]. While it could be assumed that this result was related to very short duration of diabetes, similar findings have been reported after 6 weeks [69,74], 10 weeks [69], 12 weeks [75], 16 weeks [76], 90 and 200 days of diabetes in rats [77]. Similar β-AR density has also been reported in 16-week diabetic C57BL/6 mice compared to control group [78]. Moreover, one group even reported an increased expression in two consecutive studies in female rats, both 2 weeks [79] and 3 weeks after STZ injection [80].
Table 1. Cardiac β-Adrenoceptors (β-AR) protein and mRNA levels in type 1 (T1DM) and type 2 diabetes (T2DM).

| Reference | β-AR protein (binding) | β-AR protein (Western blot) | β-AR mRNA | Species       | Sex   | Diabetes model          | Duration of diabetes |
|-----------|------------------------|------------------------------|------------|---------------|-------|-------------------------|---------------------|
| Amour et al., 2007 | n/a                    | β3-AR ↓ β1-AR ↑            | n/a        | Wistar rat    | Male  | STZ induced T1DM       | 4-week              |
| Amour et al., 2008 | n/a                    | β3-AR ↓ β1-AR ↑            | n/a        | Wistar rat    | Male  | STZ induced T1DM       | 4- and 12-week      |
| Aragno et al., 2012 | n/a                    | β3-AR ↓                    | n/a        | Wistar rat    | Male  | STZ induced T1DM       | 6-week              |
| Arioglu-Inan et al., 2013 | n/a                    | β3-AR ↑ β1-AR ↓            | SD rat     | Male          | STZ induced T1DM       | 8-week              |
| Atkins et al., 1985 | n/a                    | β-AR ↓                     | n/a        | SD rat        | Male  | STZ induced T1DM       | 2- and 4-week       |
| Austin and Chess-Williams, 1991 | β-AR ↑                | n/a                         | n/a        | Wistar rat    | Female| STZ induced T1DM       | 3-week              |
| Austin and Chess-Williams, 1992 | β-AR ↑                | n/a                         | n/a        | Wistar rat    | Female| STZ induced T1DM       | 2-week              |
| Beenen et al., 1997 | β-AR ↓                 | n/a                         | n/a        | SHR rat, WKY rat | Male  | STZ induced T1DM       | 8-week              |
| Bidasee et al., 2008 | n/a                    | β3-AR ↓ β2-AR ↓ β1-AR ↑      | n/a        | SD rat        | Male  | STZ induced T1DM       | 7-week              |
| Bilginoglu et al., 2007 | β-AR binding site ↓  | n/a                         | n/a        | Wistar rat    | Male  | STZ induced T1DM       | 5-week              |
| Bilginoglu et al., 2009 | β-AR ↓                 | n/a                         | n/a        | Wistar rat    | Male  | STZ induced T1DM       | 5-week              |
| Bitar et al., 1987 | β-AR ↓                 | n/a                         | n/a        | SD rat        | Male  | STZ induced T1DM       | 2-month             |
| Carillion et al., 2017 | n/a                    | β3-AR ↓ β2-AR ↑             | n/a        | Wistar rat    | Male  | STZ induced T1DM       | 8-week              |
| Cros et al., 1986 | β-AR n.c.               | n/a                         | n/a        | Rat           | n/a   | STZ induced T1DM       | 4-month             |
| Dincer et al., 2001 | n/a                    | β3-AR ↓ β2-AR ↓ β1-AR ↑      | n/a        | Wistar rat    | Male  | STZ induced T1DM       | 14-week             |
| Dubois et al., 1996 | β-AR ↓                 | n/a                         | n/a        | SHR rat, WKY rat | Male  | STZ induced T1DM       | 8-week              |
| Durante et al., 1989 | β-AR ↓                 | n/a                         | n/a        | Spontaneously diabetic Bio-Breeding (BB) rats | n/a   | Genetic T1DM           | 10-week             |
| Study                                      | β-AR   | n/a  | n/a  | Species       | Gender | Model Conditions                  | Duration |
|-------------------------------------------|--------|------|------|---------------|--------|-----------------------------------|----------|
| Eckel et al., 1991                        | ↓      | n/a  | n/a  | Wistar rat    | Male   | STZ induced T1DM                  | 3-week   |
| Gotzsche, 1983                             | ↓      | n/a  | n/a  | Wistar rat    | Female | STZ induced T1DM                  | 8-day    |
| Gunasekaran et al., 1993                   | ↓      | n/a  | n/a  | SD rat        | Male   | STZ induced T1DM                  | 4-week   |
| Heyliger et al., 1982                      | ↓      | n/a  | n/a  | SD rat        | Male   | STZ induced T1DM                  | 8-week   |
| Huisamen et al., 2001                      | ↓      | n/a  | n/a  | Wistar rat    | n/a    | STZ induced T1DM                  | 6, 10- and 20-week |
| Ingebretsen et al., 1983                   | ↓      | n/a  | n/a  | Albino SD rat | Male   | Alloxan induced T1DM              | 5-day    |
| Lahaye Sle et al., 2010                    | ↓      | n/a  | n/a  | Wistar rat    | Male   | STZ induced T1DM                  | 9-week   |
| Latifpour and McNeill, 1984                | ↓      | n/a  | n/a  | Rat           | n/a    | STZ induced T1DM                  | 6-month  |
| Le Douairon Lahaye et al., 2011            | ↑      | n/a  | n/a  | Wistar rat    | Male   | STZ induced T1DM                  | 9-week   |
| Lee et al., 2004                           | ↓      | n/a  | n/a  | New Zealand white rabbit | Male | Alloxan induced T1DM              | 12-week  |
| Matsuda et al., 1999                       | ↓      | n/a  | n/a  | Wistar rat    | Male   | STZ induced T1DM                  | 6-week   |
| Mishra et al., 2010                        | ↓      | n/a  | n/a  | (Ins2+/− Akita) mice | Male | Genetic T1DM                      | 12-week  |
| Monnerat-Cahli et al., 2014                | ↓      | n/a  | n/a  | Wistar rat    | Male   | STZ induced T1DM                  | 8-week   |
| Mooradian et al., 1988                     | n/c    | n/a  | n/a  | CDF (F-344) rat | Male | STZ induced T1DM                  | 6-week   |
| Myers et al., 2016                         | n/c    | n/a  | n/a  | C57BL/6 mice  | Male/Female | STZ induced T1DM                  | 16-week  |
| Nishio et al., 1988                        | ↓      | n/a  | n/a  | SD rat        | Male   | STZ induced T1DM                  | 1-, 3- and 10-week |
| Okatan et al., 2015                        | ↓      | n/a  | n/a  | Wistar rat    | Male   | STZ induced T1DM                  | n/a     |
| Reference | Treatment | β-AR | n/a | n/a | Species | Gender | Model | Duration |
|-----------|-----------|------|-----|-----|---------|--------|-------|----------|
| Plourde et al., 1991 | | β-AR ↓ | n/a | n/a | Wistar rat | Male | STZ induced T1DM | 10-day + 10-week |
| Ramanadham et al., 1983 | | β-AR ↓ | n/a | n/a | SD rat | Male | STZ induced T1DM | 4-week |
| Ramanadham and Tenner, 1983 | | β-AR ↓ | n/a | n/a | SD rat | Male | STZ induced T1DM | 4-week |
| Ramanadham and Tenner, 1986 | | β-AR ↓ | n/a | n/a | SD rat | Male | STZ induced T1DM | 1-3- and 6-month |
| Ramanadham and Tenner, 1987 | | β-AR ↓ | n/a | n/a | SD rat | Male | STZ induced T1DM | 4-week |
| Ramanadham et al., 1987 | | β-AR ↓ | n/a | n/a | SD rat | Male | STZ induced T1DM | 4-week |
| Savarese and Berkowitz, 1979 | | β-AR ↓ | n/a | n/a | SD rat | Male | STZ induced T1DM | 8-week |
| Saito et al., 1991 | | β-AR ↓ (AV node) | β-AR ↓ (IVS) | β-AR ↓ (AV node) | β-AR ↓ (IVS) | n/a | n/a | Wistar rat | Male | STZ induced T1DM | 3-week |
| Sellers and Chess-Williams, 2001 | | β-AR n.c. | n/a | n/a | Wistar rat | Male | STZ induced T1DM | 14-day |
| Sharma et al., 2008 | | β1-AR ↓ | β2-AR ↑ | n/a | Wistar rat | Male | STZ induced T1DM | 6-week |
| Sun et al., 2016 | | n/a | β1-AR n.c. | n/a | SD rat | Male | STZ induced T1DM | 8-week |
| Sundaresan et al., 1984 | | β-AR ↓ | n/a | n/a | SD rat | Male | STZ induced T1DM | 8-week |
| Stanley et al., 2001 | | β-AR ↓ | n/a | n/a | Yucatan minipig | Female | STZ induced T1DM | 11-week |
| Authors                  | β-AR Changes | n/a    | n/a    | Species          | Gender | Model of Diabetes            | Duration         |
|-------------------------|--------------|--------|--------|------------------|--------|-----------------------------|------------------|
| Sylvestre-Gervais et al., 1984 | β-AR ↓       | n/a    | n/a    | Wistar rat       | Male   | STZ induced T1DM            | 10-week          |
| Takeda et al., 1996     | β-AR n.c. (15, 18, 21, 24-day) β-AR ↓ (27-day) | n/a    | n/a    | SD rat           | Male   | STZ induced T1DM            | 15, 18, 21, 24 and 27-day |
| Tuncay et al., 2013     | β-AR n.c.    | n/a    | n/a    | Wistar rat       | Male   | STZ induced T1DM            | 12-week          |
| Uekita et al., 1997     | β-AR ↑ (3- and 14-week) β-AR n.c. (24- and 35-week) | n/a    | n/a    | CHAD hamsters    | Male/Female | Genetic T1DM | 3-, 14-, 24- and 35-week |
| Williams et al., 1983   | β-AR ↓       | n/a    | n/a    | Wistar rat       | Male   | STZ induced T1DM            | 8-week           |
| Zola et al., 1988       | β-AR n.c.    | n/a    | n/a    | New Zealand white rabbit | Male | Alloxan induced T1DM | 10–13 months |
| Daniels et al., 2010    | n/a          | na     | β1-AR n.c. | db/db mice       | Male/Female | Genetic T2DM | 10-week          |
| Dincer et al., 2003     | n/a          | n/a    | β1-AR ↓ | Human            | Male/Female | T2DM | < 5-year                  |
| Dubois et al., 1996     | β-AR ↓       | n/a    | n/a    | Zucker obese rat | n/a   | Insulin resistant diabetes | 20-week          |
| Fu et al., 2017         | n/a          | β1-AR n.c. β2-AR n.c. | n/a    | C57BL/6 mice     | Male   | HFD induced T2DM            | 8-week           |
| Garris, 1990            | β-AR n.c.    | n/a    | n/a    | db/db mice       | Female | Genetic T2DM                | 4- and 12-week   |
| Haley et al., 2015      | β-AR ↓       | n/a    | n/a    | SD rat           | Male   | HDF/low dose STZ induced T2DM | 8-week          |
| Haley et al., 2015      | β-AR n. c. (10-week) β1-AR ↓ (16-week) β2-AR ↓ (10-/16-week) β1-AR ↑ (10-/16-week) | n/a    | n/a    | ZDF rat          | Male   | Genetic T2DM                | 10- and 16-week  |
| Huisamen et al., 2001   | β-AR n.c.    | n/a    | n/a    | Zucker obese rat | n/a   | Insulin resistant diabetes model | 6-, 10- and 20-week |
| Study                      | Gender  | Model                     | Diet/Induction                      | Week(s) | 
|----------------------------|---------|---------------------------|-------------------------------------|---------|
| Jiang et al., 2015         |         | n/a                       | Zucker obese diabetic rat           | Male    |
| Kleindienst et al., 2016   | Male    | C57BL/6 mice              | HF/HS diet induced T2DM             | 12-week |
| Lamberts et al., 2014      | Male/Female | Human                     | T2DM                                | <1-year |
| Schaffer et al., 1991      | Male    | Neonatal Wistar rat       | Male Non insulin dependent diabetes | 10- and 12-month |
| Thackeray et al., 2011     | Male    | SD rat                    | HFD/low dose STZ induced T2DM       | 2- and 8-week |
| Thaung et al., 2015        | Male    | ZDF rat                   | Male Genetic T2DM                   | 20-week |
| Wang et al., 2017          | Male    | C57BL/6J mice             | Male HFD induced T2DM               | 6-month |

AV: atrioventricular, HF: high fat, HFD: high fat diet, HS: high sucrose, IVS: interventricular septum, LV: left ventricle, RA: right atrium, SD: Sprague Dawley, SHR: spontaneously hypertensive, STZ: streptozocin, T1DM: type 1 diabetes mellitus, T2DM: type 2 diabetes mellitus, WKY: Wistar Kyoto, ZDF: Zucker diabetic fatty. n.c.: no change, n/a: no data available, ↓: decreased, ↑: increased.
Subtype specific alterations of β-AR have been also reported by using radioligand binding studies in combination with subtype-selective competitors. β1- and β2-AR density has been found to be downregulated in the AV node, whereas the expression of β1- and β2-AR were decreased and increased in interventricular septum, respectively in 3-week diabetic Wistar rats [44]. Apart from the studies on rodents, β-AR density in transmural left ventricle was not altered after 12 weeks of diabetes in male pigs [81] whereas the receptor number was found to be reduced in the right atrium of 11-week diabetic female swine [82]; β-AR density was decreased after 12 weeks of Alloxan-induced diabetes in New Zealand white rabbits [83] while no alteration was found after 10–13 months of diabetes in the same model [84]. β-AR density was reported to be increased after 3 and 14 weeks in Chinese spontaneously diabetic hamsters, but unchanged after 24 and 35 weeks [85].

Protein expression of β1-AR was found to be decreased in the diabetic rat heart by using immunobLOTS [37,38,39,41,42,43,86,87,88]. β2-AR protein expression level was shown to be downregulated in diabetic (Ins2+/− Akita) mice [89]. Protein expression of β2-AR was also reduced in 14-week STZ diabetic rats [42]. Similar findings have been presented by other study groups in the same model [41,43]. On the other hand, Sharma et al. have reported upregulation of β2-AR protein in 6-week STZ diabetic rat [87]. After the presence has been demonstrated in the cardiac tissue [12], the expressional status of β2-AR has been an issue of interest. Protein expression of β2-AR was almost doubled in 14-week STZ diabetic rat heart [42]. Of note, this was the first study to report the change of all subtypes in the diabetic rat heart. The upregulation of β2-AR in the diabetic rat heart has been confirmed in the studies in STZ-diabetic rat model [36,37,39,41,43,86,87,88,90].

Consistent with the reduced protein expression level, mRNA expression of β1-AR was decreased in the diabetic rat heart [36,41,42,91]. Despite downregulation of protein expression, β2-AR mRNA expression was found to be increased in 14-week STZ diabetic rats [42]. This finding has been supported by other investigators [41,43]. In line with the change in protein expression level, mRNA expression of β2-AR was shown to increased in 14-week STZ diabetic rat heart [42]. The upregulation of β2-AR mRNA level has also determined by others in STZ-diabetic rat model [40,41].

2.1.2. mRNA and Protein Expression in Type 2 Diabetes Mellitus

β-AR protein level has been found to be unchanged after 4 and 12 weeks of diabetes in female db/db mice by using radioligand binding assay [92]. Similar β-AR protein level has been observed after 10–12 months of diabetes in a neonatal rat model of T2DM compared to control group [93]. Similarly, β-AR density was preserved in Zucker fa/fa obese rats at 6, 10, and 20 weeks of diabetes [69]. On the other hand, Dubois et al. showed that a reduced β-AR protein level in obese Zucker diabetic fatty (ZDF) rats [66].

β1-AR protein expression has found to be preserved in atrial appendages of diabetic patients [94]. However, high fat fed C57BL/6 mice did not present an alteration in the protein expression of β1-AR [95,96]. Protein expression of β1-AR was decreased in high fat fed-STZ injected rats while β2-AR density was not altered [97]. In another study, protein expression of both β1- and β2-AR were preserved whereas β3-AR were downregulated in 12-week diabetes induced by high fat/high sucrose diet and +%10 sucrose in drinking water [98]. However, Jiang et al. have demonstrated that β1- and β2-AR protein expression were reduced and β3-AR density was not altered ZDF rats compared to control [99]. Similar to these findings, β1- and β2-AR were shown to be downregulated whereas β3-AR density was preserved in high fat fed-STZ injected rats [100]. After this study, the same investigator group have determined the change of β-AR subtypes at 10 and 16 weeks of diabetes in ZDF rats. They have reported that protein expression of β1-AR was preserved at 10th week, however it was decreased at 16th week. On the other hand, β2- and β3-AR were downregulated and upregulated respectively at both time points [101]. In addition, expressional change of β-AR in left ventricle and right atrium was compared in 20-week old ZDF rats. The protein level of β1-AR and β2-AR was decreased and increased respectively in the left ventricle of diabetic animals whereas both β1- and β2-AR have been found to be upregulated in the right atrium [102].
The mRNA expression of β1- and β2-AR was decreased in the atrial appendage of diabetic patients [103]. Daniels et al. have reported that mRNA expression of β1-AR was not altered in both sexes in 10-week diabetic db/db mice [104].

2.2. β-AR Mediated Signaling Pathways

As explained in detail in section 1, due to their Gs- and/or Gi-coupled structure, stimulation of the three subtypes of the β-AR in cardiac tissue leads to activation of different downstream pathways, resulting in contraction or relaxation of cardiomyocytes. In this section, we focus on the changes in these downstream pathways and in related molecules for Gs- and Gi-coupled β-AR mediated responses separately.

2.2.1. Changes in Gs-Coupling β-Adrenoceptor Mediated Signaling Pathways in Diabetes

Stimulation of Gs-coupled β1- and β2-AR results in activation of AC-cAMP-PKA pathway and later PKA-mediated phosphorylation of several key proteins which are responsible of contraction of cardiomyocytes. Similar [82] and decreased Gs expression level [81] in cardiac tissue has been shown in T1DM Yucatan micropigs and minipigs respectively. Uekita et al. reported that Gsα subunit expression did not differ T1DM hamsters compared to the control group [85]. Basal AC activity mostly remained unchanged in diabetic heart (Table 2), but increased basal AC activity has been shown in diabetes by some investigators [85,105]. Most studies have shown that basal cAMP level in cardiac tissue has not been changed in both T1DM [54,72,106,107,109,110,111] or T2DM [112] animals. Similarly, not cardiac but plasma cAMP level has not been found different in T1DM patients compared to healthy subjects [113,114]. Decreased cAMP levels were shown in long term T1DM [69] and T2DM animals [115]. In an insulin resistant rat model, basal cardiac cAMP level was found increased at 6 weeks, unchanged at 10 weeks and decreased at 20 weeks of diabetes [69]. Interestingly, Uekita et al. have found an increased basal cardiac cAMP level in hamsters [85]. While basal cAMP level remains mostly unchanged in diabetic heart, β-AR mediated cAMP accumulation has been found decreased in some [72,106,107,112,116] but not in all studies (Table 2).
Table 2. The change in cardiac β-AR signaling pathway in T1DM and T2DM.

| Reference                        | Downstream molecule                          | Change     | Species      | Sex      | Diabetes model                  | Duration of diabetes |
|----------------------------------|---------------------------------------------|------------|--------------|----------|---------------------------------|---------------------|
| Amour et al., 2007              | G protein catalytic subunit dependent AC activity | ↓          | Wistar rat   | Male     | STZ induced T1DM                | 4-week              |
|                                  | Receptor mediated AC activity               | ↓          |              |          |                                 |                     |
|                                  | Stimulated PKA activity                     | ↓          |              |          |                                 |                     |
|                                  | NOS activity                                | ↑          |              |          |                                 |                     |
|                                  | NOS1 expression                             | ↑          |              |          |                                 |                     |
| Amour et al., 2008              | G protein catalytic subunit dependent AC activity | n.c.       | Wistar rat   | Male     | STZ induced T1DM                | 4-week              |
|                                  | Receptor mediated AC activity               | n.c.       |              |          |                                 |                     |
|                                  | Stimulated PKA activity                     | n.c.       |              |          |                                 |                     |
| Aragno et al., 2012             | p-AKT/AKT                                   | ↓          | Wistar rat   | Male     | STZ induced T1DM                | 6-week              |
| Arioglu-Inan et al., 2013       | eNOS expression                             | n.c.       | SD rat       | Male     | STZ induced T1DM                | 8-week              |
| Austin and Chess-Williams, 1991 | G protein catalytic subunit dependent AC sensitivity | ↑          | Wistar rat   | Female   | STZ induced T1DM                | 3-week              |
| Atkins et al., 1985             | Receptor mediated AC activity               | ↓          | SD rat       | Male     | STZ induced T1DM                | 4-week              |
| Beenen et al., 1997             | G protein catalytic subunit dependent AC activity | ↑ (SHR diabetic rat) | SHR rat | Male | STZ induced T1DM | 8-week |
|                                  | Receptor mediated AC activity               | ↓ (male)   | Wistar rat   | Male/Female | STZ induced T1DM | 5-week |
|                                  | Receptor mediated AC activity               | n.c. (female) |              |          |                                 |                     |
| Bilginoglu et al., 2007         | Receptor mediated AC activity               | ↓          | Wistar rat   | Male     | STZ induced T1DM                | 5-week              |
|                                 | Receptor mediated AC activity               | n.c.       |              |          |                                 |                     |
| Bockus and Humphries, 2015      | Basal cAMP level                            | n.c.       | C57BL/6J mice| Male     | STZ induced T1DM                | 4-month             |
|                                 | Basal PKA activity                          | n.c.       |              |          |                                 |                     |
|                                 | Stimulated PKA activity                     | ↓          |              |          |                                 |                     |
| Das, 1973                       | Basal cAMP level                            | n.c.       | SD rat       | Male     | STZ induced T1DM                | 7-day               |
|                                 | Basal AC activity                           | n.c.       |              |          |                                 |                     |
| El-Hage et al., 1985            | Basal cAMP level                            | n.c.       | CDI mice     | Male     | Alloxan induced T1DM             | 10-day              |
|                                 | Receptor mediated cAMP level                | n.c.       | C57BL/Ksjj mice| Male | Genetic diabetes | 10-day |
| Gotzsche, 1983                  | Basal cAMP levels                           | ↓          | Wistar rat   | Female   | STZ induced T1DM                | 8-day               |
| Study                          | Basal AC activity | G protein catalytic subunit dependent AC activity | Receptor mediated AC activity | Basal cAMP level | Receptor mediated cAMP level | Species                | Sex        | Induction Type          | Duration        |
|-------------------------------|-------------------|-----------------------------------------------|-------------------------------|-----------------|-------------------------------|------------------------|-----------|------------------------|-----------------|
| Huisamen et al., 2001         | n.c. (6-week)     | n.c. (10-week)                                | n.c. (20-week)                | n.c. (6-week)   | n.c. (6-week)                 | Wistar rat            | n/a       | STZ induced T1DM        | 6-, 10- and 20-week |
| Ingebretsen Jr et al., 1981   |                   |                                               |                               |                 |                               | SD rat                 | Male      | Alloxan induced T1DM   | n/a             |
| Ingebretsen et al., 1983      |                   |                                               |                               |                 |                               | SD rat                 | Male      | Alloxan induced T1DM   | 5-day           |
| Study                          | Effect | Species     | Gender | Disease Model          | Duration |
|-------------------------------|--------|-------------|--------|------------------------|----------|
| Cells 2020, 9, 2548           |        |             |        |                        |          |
| G protein catalytic subunit   |        |             |        |                        |          |
| dependent AC activity        | n.c.   |             |        |                        |          |
| G protein dependent AC        | n.c.   |             |        |                        |          |
| activity                      | n.c.   |             |        |                        |          |
| Receptor mediated AC activity| n.c.   |             |        |                        |          |
| Kayki-Mutlu et al., 2014     | ↑      | SD rat      | Male   | STZ induced T1DM       | 8-week   |
| Gia2 expression               |        |             |        |                        |          |
| eNOS expression              | n.c.   |             |        |                        |          |
| Le Douairon Lahaye et al., 2011| ↑      | Wistar rat  | Male   | STZ induced T1DM       | 9-week   |
| NOS1 expression              |        |             |        |                        |          |
| Menahan et al., 1977         | ↓      | Rat         | n/a    | Alloxan induced T1DM   | 13–14 days |
| Basal AC activity            | n.c.   |             |        |                        |          |
| G protein dependent AC        | ↓      |             |        |                        |          |
| activity                      |        |             |        |                        |          |
| Receptor mediated AC activity| ↓      |             |        |                        |          |
| Michel et al., 1985          | ↓      | Wistar rat  | Male   | STZ induced T1DM       | 4-month  |
| Basal AC activity            | n.c.   |             |        |                        |          |
| G protein dependent AC        |        |             |        |                        |          |
| activity                      | n.c.   |             |        |                        |          |
| Receptor mediated AC activity| ↓      |             |        |                        |          |
| Miller Jr, 1984              | ↓      | SD rat      | Male   | Alloxan induced T1DM   | 3–7 days |
| Basal cAMP level             | n.c.   |             |        |                        |          |
| Receptor mediated cAMP level | ↓      |             |        |                        |          |
| Mooradian et al., 1988       |        | CDF (F-344) rat | Male   | STZ induced T1DM       | 6-week   |
| G protein catalytic subunit   | n.c.   |             |        |                        |          |
| dependent AC activity        |        |             |        |                        |          |
| Receptor mediated AC activity| n.c.   |             |        |                        |          |
| Nishio et al., 1988          |        | SD rat      | Male   | STZ induced T1DM       | 10-week  |
| Basal AC activity            | n.c.   |             |        |                        |          |
| G protein catalytic subunit   |        |             |        |                        |          |
| dependent AC activity        | n.c.   |             |        |                        |          |
| Receptor mediated AC activity| ↓      |             |        |                        |          |
| Plourde et al., 1991         |        | Wistar rat  | Male   | STZ induced T1DM       | 10-day + 10-week |
| G protein catalytic subunit   | n.c.   |             |        |                        |          |
| dependent AC activity        | n.c.   |             |        |                        |          |
| Receptor mediated AC activity| n.c.   |             |        |                        |          |
| Ramanadham and Tenner, 1987  |        | SD rat      | Male   | STZ induced T1DM       | 4-week   |
| G protein catalytic subunit   | n.c.   |             |        |                        |          |
| dependent AC activity        |        |             |        |                        |          |
| Roth et al., 1995            |        | Yucatan minipig | Male   | STZ induced T1DM       | 12-week  |
| Basal AC activity            | n.c.   |             |        |                        |          |
| Study                         | Basal PKA activity | p-AKT expression | β2-Gs coupling | β2-Gi coupling | Species   | Gender | Model/Condition | Time | Remarks |
|-------------------------------|--------------------|------------------|----------------|----------------|-----------|--------|----------------|------|---------|
| Sharma et al., 2008          | n.c.               | ↓                | n.c.           | n.c.           | Wistar rat | Male   | STZ induced T1DM | 6-week |         |
| Sharma et al., 2011          | n.c.               | ↓                |               |               | Wistar rat | Male   | STZ induced T1DM | 6-week |         |
| Shao et al., 2009             | n.c.               | ↓                |               |               | SD rat    | Male   | STZ induced T1DM | 7-week |         |
| Smith et al., 1984            | n.c.               |                 |               |               | SD rat    | Male   | STZ induced T1DM | 8–9 weeks |         |
| Smith et al., 1997            | ↑                  | ↑                |               |               | SD rat    | Male   | STZ induced T1DM | 8-week |         |
| Srivastava and Anand-Srivastava, 1985 | ↑                  |                 |               |               | SD rat    | Male   | STZ induced T1DM | 5-day |         |
| Study                        | Basal AC activity                                      | G protein catalytic subunit dependent AC activity | G protein dependent AC activity | Receptor mediated AC activity | Gs expression | Gi expression | Yucatan micropig | Gender | STZ induced T1DM | Duration  |
|------------------------------|--------------------------------------------------------|--------------------------------------------------|--------------------------------|-------------------------------|---------------|---------------|------------------|--------|------------------|-----------|
| Stanley et al., 2001         | n.c.                                                   | ↓                                                | ↓ n.c.                         | n.c.                          |               |               | Female            |        | STZ induced T1DM | 11-week  |
| Sundaresan et al., 1984      | Basal cAMP levels                                     | n.c.                                             | n.c.                           | n.c.                          |               |               | SD rat           | Male   | STZ induced T1DM | 2-month  |
| Tamada et al., 1998          | G protein catalytic subunit dependent AC activity      | ↓                                                |                                |                               |               |               | Wistar rat       | Male   | STZ induced T1DM | 4–6 weeks|
| Trovik et al., 1994          | Plasma cAMP level                                     | n.c.                                             |                                |                               |               |               | Human            | Male/Female | Insulin dependent T1DM | n/a       |
| Tuncay et al., 2013          | p-PKA/PKA                                              | ↑                                                |                                |                               |               |               | Wistar rat       | Male   | STZ induced T1DM | 12-week  |
| Uekita et al., 1997          | Basal AC activity                                     | ↑ (14-week)                                      | ↑ (14-/24-week)                |                               |               |               | CHAD hamsters    | Male/Female | Genetic T1DM     | 3-, 14-, 24- and 35-week |
| Vadlamudi and McNeill, 1983  | Basal cAMP level                                      | n.c. (3-day)                                      |                                |                               |               |               | Wistar rat       | Female | STZ and/or alloxan induced T1DM | 3-day, 100–120 days |

Legend:
- **STZ induced T1DM**: STZ-induced type 1 diabetes mellitus
- **SD rat**: Sprague-Dawley rat
- **Wistar rat**: Wistar rat
- **Yucatan micropig**: Yucatan micro pig
- **Human**: Human
- **Genetic T1DM**: Genetic type 1 diabetes mellitus
- **Basal cAMP level**: Basal cyclic adenosine monophosphate
- **Receptor mediated cAMP level**: Receptor-mediated cyclic adenosine monophosphate
- **G protein catalytic subunit dependent AC activity**: G protein catalytic subunit-dependent adenyl cyclase activity
- **G protein dependent AC activity**: G protein-dependent adenyl cyclase activity
- **Receptor mediated AC activity**: Receptor-mediated adenyl cyclase activity
- **Gs expression**: Gs expression
- **Gi expression**: Gi expression
- **↑**: Increased
- **↓**: Decreased
- **n.c.**: Not changed
| Basal cAMP level | Receptor mediated cAMP level |
|------------------|-----------------------------|
| n.c. (100–120 days) | n.c. (100–120 days) |

| Receptor mediated cAMP level | ↓ | Wistar rat | Male | STZ induced T1DM | 4–5 weeks |
|------------------------------|---|------------|------|------------------|---------|
| Stimulated PKA activity      |   |            |      | STZ induced T1DM | 6-week  |
| Receptor mediated PKA activity | ↓ | Wistar rat | Male | STZ induced T1DM | 6-week  |
| Gi expression                | n.c. | C57BL/6 mice | Male | HFD induced T2DM | 8-week  |
| p-TnI/TnI                    | n.c. |            |      |                  |         |
| p-PLN<sup>swt</sup>/PLN      | n.c. |            |      |                  |         |
| Receptor mediated p-PLN<sup>swt</sup>/PLN | ↓ | C57BL/6 mice | Male | HFD induced T2DM | 8-week  |

| Plasma cAMP level | ↓ | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
|------------------|---|-------|------|------------------------------------------|-------------|
| Basal AC activity | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| G protein catalytic subunit dependent AC activity | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| Receptor mediated AC activity | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| Basal AC activity | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| G protein catalytic subunit dependent AC activity | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| Receptor mediated AC activity | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| Basal AC activity | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| G protein catalytic subunit dependent AC activity | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| Receptor mediated AC activity | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| Basal cAMP level | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| Receptor mediated cAMP level | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| Basal cAMP level | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| Receptor mediated cAMP level | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| Basal cAMP level | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |
| Receptor mediated cAMP level | n.c. | Human | Male | Insulin dependent juvenile onset diabetes | 12–16 years |

| Zucker obese rat | na | Insulin resistant diabetes | 6-, 10- and 20-week |
| Study: Jiang et al., 2015 | G protein catalytic subunit dependent AC activity | n.c. (6-week) | Zucker obese diabetic rat | Male | Genetic T2DM | 15-week |
|--------------------------|-----------------------------------------------|----------------|--------------------------|------|--------------|--------|
|                          | Stimulated PKA activity                       | ↓              |                          |      |              |        |

| Study: Kleindienst et al., 2016 | eNOS expression | ↑ | C57BL/6 mice | Male | HF/HS diet induced T2DM | 12-week |
|---------------------------------|------------------|---|--------------|------|------------------------|--------|
|                                 | p-eNOS<sup>Ser1177</sup> expression | ↑ |              |      |                        |        |
|                                 | nNOS expression | ↓ |              |      |                        |        |
|                                 | p-eNOS<sup>Thr495</sup> expression | ↑ |              |      |                        |        |
|                                 | iNOS expression | ↓ |              |      |                        |        |

| Study: Schaffer et al., 1991   | Receptor mediated AC activity | n.c. | Neonatal Wistar rat | Male | Non insulin dependent diabetes | 10- and 12-month |
|--------------------------------|-------------------------------|------|---------------------|------|-------------------------------|-----------------|
|                                | Receptor mediated cAMP level | n.c. |                      |      |                               |                 |
|                                | Na<sup>-</sup>-K<sup>+</sup> ATPase activity | ↓ |              |      |                               |                 |

| Study: Song et al., 2008       | iNOS expression | ↑ | ZDF rat | Male | Genetic T2DM | 20-week |
|--------------------------------|-----------------|---|---------|------|--------------|--------|
|                                | iNOS activity   | ↑ |          |      |              |        |

| Study: Wang et al., 2017       | Basal cAMP level | ↓ | C57BL/6J mice | Male | HFD induced T2DM | 6-month |
|--------------------------------|------------------|---|--------------|------|------------------|--------|
|                                | Gi expression    | n.c. |                |      |                  |        |
|                                | p-PLN/PLN        | ↓ |                |      |                  |        |
|                                | p-AKT expression | ↑ |                |      |                  |        |
|                                | p-TnI/TnI        | ↓ |                |      |                  |        |

| Study: West et al., 2019       | Basal cAMP level | n.c. | C57BL/6J mice | Male | HFD induced T2DM | 4.5–5 months |
|--------------------------------|------------------|------|--------------|------|------------------|--------------|
|                                | Receptor mediated cAMP level | n.c. |                |      |                  |        |
|                                | Basal cGMP level | n.c. |                |      |                  |        |
|                                | Receptor mediated cGMP level | n.c. |                |      |                  |        |
|                                | Basal PKA activity | ↓ |                |      |                  |        |
|                                | Receptor mediated PKA activity | ↓ |                |      |                  |        |
|                                | p23–24/Total TnI | ↓ |                |      |                  |        |
Alteration in β-AR mediated AC activity in diabetes has been evaluated by using non-selective β-AR agonist isoprenaline. There are studies which show similar AC activity [50,54,74,81,86] or decreased AC activity [37,55,60,62,71,72,81,105,117,118,119] or increased AC activity [85] in response to β-AR agonist stimulation in T1DM animals. Schaffer et al. showed similar receptor-mediated AC activity in a non-insulin-dependent diabetes model [93]. Regardless of duration of diabetes, receptor-mediated AC activity has been found similar in both T1DM and T2DM diabetic rats compared to control group [69]. Bilginoglu et al. showed that β-AR mediated AC activity attenuated in male but remained unchanged in female T1DM rats [70]. Heterotrimeric G protein-dependent AC activity has been evaluated in studies of diabetes. Stimulation of heterotrimeric G protein has been shown to result in similar AC activity [50,62,117,118] or decreased AC activity [81,82,105,119] or increased AC activity [85] in diabetic animals compared to control group. Forskolin is a direct AC stimulator and often used to investigate intrinsic AC activity. Forskolin-induced AC activity was found similar in both T1DM [50,58,60,74,81,86,117] and T2DM animals [69] compared to the control group. On the other hand, attenuation of forskolin-induced AC activity in T1DM [37,82,105,120] and in T2DM [99] has been shown in other studies. Similarly, studies that found increased basal AC activity found increased AC activity in diabetes in response to forskolin [67,85]. Austin and Chess-Williams showed increased AC sensitivity to forskolin in T1DM [80].

Similar basal cardiac PKA activity has been shown in T1DM rats [87,110] and in T2DM mice [112]. Shao et al. also showed decreased basal PKA activity in T1DM rats [121]. Stimulation of PKA using by either 8-Bromo-cAMP or dibutyryl-cAMP mostly been shown to cause reduced activity in both T1DM and T2DM [37,99,110,120,122]. However, similar PKA activity was observed in diabetic animals in response to cAMP-derivative stimulation as in control animals [86,121]. In addition to this, isoprenaline stimulated PKA activity was decreased in T1DM rat [122] and T2DM mice [112]. While phosphorylation of PKA was not found different in 6-week T1DM rats [123], phosho-PKA/PKA ratio has been shown to increase when the duration of diabetes reached 12 week [75].

Changes in PKA-dependent phosphorylation of contractile proteins may also be responsible for diabetes related cardiac dysfunction. Alterations in PKA mediated phosphorylation of phospholamban (PLN), ryanodine receptor (RyR) and troponin I (TnI) proteins due to diabetes have been investigated in the studies which we used to generate this review. p-PLN expression was shown to decrease in T1DM rats [75]. Decreased p-PLN/PLN ratio was also found in T2DM animals [112,115]. p-PLN/PLN ratio has been shown to unchanged in T2DM mice, however isoprenaline stimulation caused a decrease in this ratio [96]. The p-RyR/RyR ratio was decreased in T1DM rats [75]. On the other hand, p-RyR expression at both Ser2814 and Ser2808 sites was found to increase in T1DM rats [121]. Whereas, decreased p-TnI/TnI ratio was shown in T2DM mice [112,115], unchanged p-TnI/TnI ratio was found by other research group [96] in the same diabetes model. While modulation of ion channel function, mostly various types of K+ and Ca++ channels, is an important effector pathway of cardiac β-AR, our search did not identify studies describing alterations of such coupling in diabetes; therefore, they are not discussed here.

2.2.2. Changes in Gα-Coupling β-Adrenoceptor Mediated Signaling Pathways in Diabetes

Different from β1-AR, stimulation of β2-AR also activates the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway, which protects cardiac cells against apoptosis through its Gı coupling [124]. Unlike β1- and β2-AR, stimulation of β3-AR activates NOS-NO-cGMP-PKG pathway through its Gı coupling, resulting in relaxation of cardiomyocytes. It has been shown that the Gı expression level [82,96,115] and Gα subunit expression level [85] do not change in diabetes. On the other hand, increased Gı expression level and Gı/Gı ratio was found in T1DM Yucatan minipigs [81]. Kayki-Mutlu et al. have reported increased Gı2 subunit expression level in T1DM rats [40].

Coupling of cardiac β2-AR with the Gı and Gα-protein did not differ between T1DM and control rats [87]. It has been shown that AKT phosphorylation [123], p-AKT protein expression [87] and p-AKT/AKT ratio [38] were reduced in T1DM animals. However, Wang et al. found an elevated p-AKT expression level in T2DM [115]. In T2DM, β2-AR activate cGMP-PKG signaling through a Gı-coupled pathway, which has anti hypertrophic effect on heart [112]. In this study, basal cardiac cGMP levels
did not differ between diabetic and control animals, but an increased cGMP level has been observed in response to isoprenaline stimulation in diabetic animals [112].

There are few studies investigating changes in β-AR mediated downstream molecules in diabetes. Basal cardiac cGMP levels were found to be unchanged in T1DM rats compared to their age-matched controls [106]. Increased NOS activity [37] and NOS1 protein expression were reported in T1DM rats [37,90]. Similarly, increased NO and cNOS expression level and iNOS induction were shown in T1DM rats [125]. However, eNOS expression did not change in T1DM rats after 8 weeks of diabetes [36,40]. Contrary to these findings, Kleindienst et. al. found increased eNOS and p-eNOS, both at Ser1177 and Thr495 site, expression in T2DM mice compared to the control group [98]. Moreover, it was found that nNOS expression decreased and iNOS expression increased in diabetic animals [98]. Consistent with this study, increased iNOS expression and activity were also found in genetically T2DM rats [126], β-AR mediated activation of Na+/K+ pump has beneficial effects on heart in pathological situation [127]. Unchanged Na+-K+ ATPase activity was found in T1DM hamsters [85]. However, Schaffer et al. showed that diabetes could cause reduction in Na+/K+ ATPase activity in long term [93].

2.3. The Inotropic and Chronotropic Response to β-AR Stimulation in the Diabetic Heart

The impact of diabetes on β-AR mediated cardiac function has been widely investigated as β-AR are the essential component of cardiac contraction [128]. The inotropic and chronotropic responses to β-AR stimulation in the diabetic heart has been determined both in vivo and in vitro. Cardiomyocytes, atrial or ventricular tissue or papillary muscle or whole heart preparations have been used in in vitro studies. The studies have mostly been conducted on rats but also been done on mice, swine, rabbit and humans both in T1DM and T2DM.

In this section, β-AR functional changes in cardiac tissue is described, firstly, the contraction and/or relaxation responses obtained in in vitro studies and Ca++-mediated responses, if any, and then the responses obtained from in vivo studies in T1DM and T2DM separately.

2.3.1. Type 1 Diabetes Mellitus

Ventricular myocytes have been isolated from Wistar rats and the isoprenaline-induced contractile response was not affected after 24 h of hyperglycemic exposure [129]. In line with this result, it has been shown that hyperglycemia has no detrimental effect on ventricular cardiomyocytes after isoprenaline stimulation [130]. The isoprenaline-mediated effect on peak shortening was reduced in ventricular myocytes isolated from STZ-diabetic mice [131]. Different from these studies, rate of contraction and relaxation time after isoprenaline treatment were augmented in cardiomyocytes of diabetic Ins2−/−Akita mice [89].

The inotropic effect of isoprenaline was attenuated in chronic diabetic rat atria [132,133,134]. Reduced inotropic effect in right atria has been also confirmed despite absence of a relevant change in the chronotropic effect [135]. Decreased inotropic effect to β-AR stimulation has also been observed in the left atria [136]. Reduced chronotropic effect of β-AR agonists has been reported by several investigators [132, 133, 137, 138]. While isoprenaline stimulated chronotropic response has been found to be impaired in right atria of acute and chronic diabetes [51], isoprenaline induced inotropy has mostly been found unaltered in the studies by same study group [56,57,58,139] except for the one study with enhanced inotropic response [51]. The idea of increased inotropic effect of isoprenaline has been supported in the left atria of 2-week diabetic rats which was reversed after 12 weeks of the pathology [140]. Preserved inotropic effect of β-AR stimulation has been also shown in the right and left atria of spontaneously diabetic Bio-breeding (BB) rats [61]. Isoprenaline induced chronotropic effect of the right atrium was not different in diabetic Yucatan minipigs [82], whereas it was found to be depressed in diabetic rats [141].

The discrepancy between the functional studies are also present in the isolated ventricular tissue. A reduced maximum response to isoprenaline in right ventricular strips [142] and decreased contractile response to isoprenaline in ventricular tissue [143] have been observed in diabetic rats. Isoprenaline-induced developed tension of right ventricular strip was numerically decreased in
alloxan diabetic rabbit, but this effect has not been found to be statistically significant [84]. Gunasekaran et al. have demonstrated that isoprenaline induced maximum response was not altered in right ventricular strip of 4-week diabetic rats [64]. On the other hand, Wald et al. have demonstrated that force of contraction was increased in the ventricle of STZ diabetic rats [144]. β-AR responsiveness has been also investigated by using papillary muscles. Contractile force generation by isoprenaline stimulation was reduced in the papillary muscle in 8-week diabetic rats [48]. This finding has been confirmed by other groups [37,38,39,145,146,147,148,149,150] including our group [36]. On the other hand, preserved contractile response to isoprenaline in the papillary tissue has also been reported in long term diabetes [79]. Preserved positive lusitropic effect in response to isoprenaline administration, in the presence of β-AR antagonist and NOS inhibitor, in papillary muscle has also been reported in both acute and chronic diabetes [86]. We showed blunted relaxation response to BRL 37344, a mixed β2/β3-AR agonist, in the papillary muscle [36]. The sensitivity to noradrenaline in right atria or ventricular papillary muscle was not changed in diabetic rabbits [151]. However, Austin et al. showed increased sensitivity to isoprenaline of both left atria and papillary muscle without any significant alteration in the maximum response to isoprenaline in 14-day diabetic female rat [152].

Whole heart preparation has been used in some of the studies. The peak response to isoprenaline stimulation was not changed in Langendorff perfused hearts in 9-week diabetic rats [88]. Maximum inotropic response to isoprenaline was found to be increased in female rat heart while it was not changed in male rats [70]. Preserved responses to β-AR agonists were also found by others [153,154]. Comparable results in maximum increase in developed tension, heart rate, +dp/dt and -dp/dt after isoprenaline stimulation were observed among the groups although the increase at submaximal doses of the drug was greater in the diabetic group [73]. On the other hand, using Langendorff or working heart preparation, an impaired response to β-AR stimulation was also shown [71,106,125,155,156,157]. Unchanged +dp/dt in response to isoprenaline stimulation was shown by using working heart preparation in both alloxan and STZ diabetic female rats. However, -dp/dt was depressed at both acute and chronic phase of diabetes in the same study [158]. In some of the studies, the functional response to β-AR stimulation has been subtype specifically investigated. Dobutamine induced β1-AR mediated inotropic effect was enhanced in the diabetic heart whereas salbutamol-induced β2-AR mediated contractility was comparable in STZ diabetic spontaneously hypertensive (SHR) rats compared to control group [67]. β1- and β2-AR mediated inotropic effect was preserved and reduced in STZ diabetic rat heart, respectively. On the other hand, BRL 37344 stimulated β-AR mediated relaxation was increased in Langendorff perfused hearts [41]. Augmented relaxation response to BRL 37344 in the same diabetic model has been also shown by our group [40].

β-AR mediated responses have been also evaluated by the change in Ca++ transients. Increase in Ca++ transient amplitude as a response to orciprenaline was lower in cardiomyocytes isolated from 1- and 6- week diabetic rats [159]. Isoprenaline induced effect in Ca++ transient and cell shortening was impaired in ventricular myocytes isolated from 4–6 week diabetic rats [120]. Parallel to these findings, blunted [Ca++]i change in response to isoprenaline was reported in diabetic cells [122].

There are relatively fewer studies which have investigated β-AR mediated in vivo in T1DM. In vivo cardiac parameters such as rate of contraction/relaxation and heart rate were attenuated after isoprenaline treatment in STZ diabetic mice [110]. Similarly, isoprenaline induced in vivo inotropic response was decreased in 16-week diabetic mice [78]. +dp/dt was depressed after isoprenaline stimulation in 4-week diabetic rats whereas it was preserved in 2-week diabetic group [55]. Both +dp/dt and -dp/dt and amplitude of response were reduced after in vivo isoprenaline administration in 7-week diabetic rats [43]. Decreased response to in vivo β-AR stimulation in the diabetic rat heart has been also shown by others [37,39,75,91,160,161,162,163,164,165,166]. Depressed isoprenaline stimulated response has also been shown in alloxan diabetic rabbits [83]. However, unaltered or enhanced inotropic/lusitropic response to in vivo β-AR stimulation have been also reported. Amour et al. have demonstrated that dobutamine induced positive lusitropic effect was not changed after 4 or 12 weeks of diabetes despite diastolic dysfunction [86]. Similarly, in vivo β-AR induced effect was well preserved in female diabetic rats [167]. On the other hand, dobutamine induced contractile
function was found to be increased in diabetic rats [168]. However, the effect of dobutamine on in vivo cardiac parameters were comparable between control and STZ-diabetic Yucatan minipigs [169]. There are also few studies which have determined the change in β-AR mediated effect in the human heart. β-AR sensitivity was increased in insulin dependent diabetic patients [170]. On the other hand, it has been found that sensitivity to isoprenaline stimulation was reduced in insulin dependent diabetic patients with hypoglycemic unawareness [114,171,172]. However, epinephrine induced increase in heart rate was demonstrated to be greater in diabetic patients with autonomic neuropathy [113]. On the other hand, the effect of in vivo noradrenaline administration on cardiac parameters was comparable in control and diabetic patients [173].

2.3.2. Type 2 Diabetes Mellitus

As mentioned in the previous sections, T2DM animal models have received attention because of their greater translational value. Parallel to the studies in T1DM models, mostly rats, but also mice have been used in studies on T2DM.

Isoprenaline-stimulated sarcomere shortening in cardiomyocytes isolated from high fat fed mice was not found different compared to control cardiomyocytes [174]. Isoprenaline induced chronotropic response was significantly increased in atria from fructose and sucrose fed rats which presents an insulin resistance model [175]. β-AR mediated cardiac function in right atria from was not found different among diabetic and healthy subjects [138].

The inotropic effect of isoprenaline was not altered in left ventricular trabecular muscle in neonatal non-insulin-dependent diabetes model [143]. Similarly, trabeculae tissue was found to be unresponsive to dobutamine stimulation in T2DM individuals [94]. Positive inotropic effect of isoprenaline was slightly and markedly decreased in papillary muscle from Zucker obese and ZDF rats, respectively [99].

β-AR mediated response on contraction and relaxation was impaired in ZDF rats by using the Langendorff heart preparation. It has been suggested that β1-AR is the main subtype which regulates heart function in both healthy and diabetic rats whereas β2-AR has an indirect influence on β-AR mediated responses in the diabetic heart [176]. Dobutamine induced chronotropic effect was reduced in unpaced hearts despite of preserved inotropic effect in whole heart in ZDF rats. Of note, contractility in diabetic groups was decreased when the heart rate is set to 300 bpm with pacing [102]. Impaired contraction and relaxation response to isoprenaline stimulation has been also shown in isolated perfused heart in neonatal noninsulin dependent diabetes model [93]. Left ventricular developed pressure after BRL 37344 stimulation was determined to be significantly increased by using Langendorff heart preparation in high fat fed mice [98].

The in vivo effect of isoprenaline was attenuated in high fat fed rats [96,112,115]. The in vivo inotropic response to dobutamine stimulation was reduced in ZDF rats [102]. Similar findings were reported by Song et al. in the same model [126]. In line with these findings, in vivo inotropic and lusitropic effect of dobutamine was found to be reduced in db/db mice [104]. However, Takada et al. have demonstrated that positive inotropic response to in vivo β-AR stimulation was similar between control and OLETF diabetic rats [177]. This finding was confirmed in diabetic individuals. Both chronotropic and inotropic response to β-AR stimulation was found to be unaffected in this study [178]. Chronotropic response to dobutamine stimulation was decreased in conscious ZDF rats [179]. Chronotropic effect of isoprenaline was augmented in ZDF rats and it has been implicated that β-AR is the main subtype to modulate chronotropic effect in control and diabetic animals [180]. Furthermore, heart rate was not altered in db/db diabetic mice after a 2-h isoprenaline administration [111].

3. Discussion

Cardiovascular complications are the major risk factor for mortality in individuals with diabetes [181]. It is important to understand underlying mechanisms that contributes to diabetes-induced cardiac dysfunction to allow researchers and physicians to prevent or treat it. Sympathetic system overactivity is well-known characteristic of diabetes. Thus, it seems logical that sympathetic
overdrive leads changes in β-AR mediated responses such as cardiac contraction and relaxation. This is an issue of interest which has been investigated by several study groups for many years. However, there are many inconsistent findings in the literature which makes it difficult to properly interpret the existing data. Moreover, the literature is dominated by studies from animal models, which may or may not be representative for the human situation. The conflicting results from the animal studies make it even harder to extrapolate to humans. Therefore, more human studies are urgently needed.

Two additional under-investigated areas emerged from our search: There is little data on alterations of β-AR coupling to ion channels in the diabetic heart and on differential regulation within the heart, for instance ventricular tissues vs. conduction system.

Most investigators have reported a decreased expression of β-AR by using radioligand binding studies in rodent models of T1DM, but a smaller number of studies did not confirm such changes, and an increased expression has been reported in two studies from one group of investigators [79,80]. The balance of these findings indicates that expression is decreased, primarily due to a reduction in β1- and β2-AR and less if any in β3-AR as explained in section 2.1. Similarly to radioligand binding studies, there are different findings about changes in subtype specific changes in β-AR by using immunoblotting by different study groups. However, changes in same subtype could differ regarding the different cardiac tissue that is used [102] or duration of diabetes [101] in the same study. There are two studies which have reported both protein and mRNA level of β-AR in diabetic heart [41,42]. In both studies, the mRNA expression of β1-AR and β3-AR was found to be decreased and increased respectively, consistent with the protein level. However, it was found that the β2-AR mRNA level was increased despite the reduced β2-AR protein level, and this has been referred to the sensitization of the receptor [42]. While not confirmed in T2DM models, an increased expression of β3-AR is one of the most consistent findings in the STZ model of T1DM. Similarly, upregulation of β3-ARs have been reported in the heart failure. In both human [29] and canine cardiomyocytes [182], the expression of β3-ARs was found to be increased. On the other hand, these studies have indicated conflicting results about the β1-AR mediated relaxation in the failed heart. The response to β1-AR stimulation was blunted in the human heart [29] while it was increased in the canine heart [182]. It has been suggested that this discrepancy may have been caused by several factors such as interspecies differences, the severity of the disease, tissue type or different measurement technique of the contractility [182]. β3-ARs have been also related to a protective role in cardiac ischemia and reperfusion injury. The beneficial effects due to β3-AR stimulation have been demonstrated in this pathology [183,184]. Of note, the favorable effect mediated by β3-AR stimulation in ischemia and reperfusion injury was seen only in control rats not in high fat high sucrose (HFS) fed mice [98]. This result has been linked to reduced expression of β3-ARs in HFS mice. These findings imply that the expression or functional changes of β3-ARs may contribute to the cardiac pathologies.

Cardiac β-AR post-receptor signaling pathway regulation may contribute to changes in β-AR responsiveness in diabetes. Supporting that, reduced β-AR responsiveness has been reported despite unchanged β-AR protein and/or mRNA level in diabetic state in some studies [96,115]. However, there are few studies which have linked the changes in β-AR subtype in molecular level and related downstream molecules and receptor mediated functional response [36,40,96,115]. Some studies have investigated the changes in the protein expression of β-AR and concomitant signaling pathway molecules, with [60,82] or without [54,81] functional response studies. And some of them have evaluated changes in signaling pathway molecules and further contraction and/or relaxation responses [110,111]. Studies investigating the cardiac β-AR signaling pathway have also revealed quite different results (Table 2). Conflicted findings have been reported on the same downstream molecule in the studies which have been done by same study group with the very same experimental approach despite similar findings on β-AR protein expression changes [37,86].

Same as the molecular changes in β-AR, in vivo and in vitro studies have yielded different results. Increased and decreased response to the same agonist, in the whole heart [40] and in the papillary muscle [36], respectively, was also shown by our study group. It has been suggested in the studies that discrepancies in findings may have resulted from experimental approach such as duration of the diabetes or the cardiac section that was used in the study. However, to interpret
existing data by attributing to single or several variables cannot go beyond an assumption, considering the existence of such inconsistent and different findings.

In conclusion, since the late 1970s, diabetes related changes in cardiac β-AR have attracted attention of several researchers but their findings are controversial. At the molecular level, antibody-based approaches are troubled by low target selectivity and in general studies have a very poor reproducibility rate. The studies primarily have been conducted on rodent cardiac tissues and few have been done in other species including humans. In preclinical studies, mostly male animals have been preferred, there are relatively fewer studies which have been done in female animals. Even though 90% of diabetic patients have T2DM [185], more studies have been done on T1DM animal models. Apart from inconsistent findings, these are other obstacles to interpret and generalize findings to the clinical stage. Thus, it is unlikely to make a general interpretation and extrapolation regarding to the studies which have been done so far. We think that investigators who do or will work on cardiac β-AR in diabetes should be aware of the inconsistency in the literature. More studies are needed to come through an overall conclusion about the role of β-AR in diabetic cardiac dysfunction.

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**References**

1. (WHO), W.H.O. Diabetes. 2020. https://www.who.int/news-room/fact-sheets/detail/diabetes. (Accessed on 07/25/2020).
2. Cho, N.H.; Shaw, J.E.; Karuranga, S.; Huang, Y.; da Rocha Fernandes, J.D.; Ohlrogge, A.W.; Malanda, B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res. Clin. Pract.* **2018**, *138*, 271–281.
3. Chen, R.; Ovbiagele, B.; Feng, W. Diabetes and Stroke: Epidemiology, Pathophysiology, Pharmaceuticals and Outcomes. *Am. J. Med. Sci.* **2016**, *351*, 380–386.
4. Haffner, S.M.; Lehto, S.; Ronnemaa, T.; Pyorala, K.; Laakso, M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl. J. Med.* **1998**, *339*, 229–234.
5. Rosano, G.M.; Vitale, C.; Seferovic, P. Heart Failure in Patients with Diabetes Mellitus. *Card. Fail. Rev.* **2017**, *3*, 52–55.
6. Action to Control Cardiovascular Risk in Diabetes Study. Effects of intensive glucose lowering in type 2 diabetes. *N Engl. J. Med.* **2008**, *358*, 2545–2559.
7. Group, A.C.; Patel, A.; MacMahon, S.; Chalmers, J.; Neal, B.; Billot, L.; Woodward, M.; Marre, M.; Cooper, M.; Glasziou, P.; et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl. J. Med.* **2008**, *358*, 2560–2572.
8. Duckworth, W.; Abraira, C; Moritz, T.; Reda, D.; Emanuele, N.; Reaven, P.D.; Zieve, F.J.; Marks, J.; Davis, S.N.; Hayward, R.; et al. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl. J. Med.* **2009**, *360*, 129–139.
9. Bylund, D.B.; Eikenberg, D.C.; Hieble, J.P.; Langer, S.Z.; Lefkowitz, R.J.; Minneman, K.P.; Molinoff, P.B.; Ruffolo RR, J.r.; Trendelenburg, U. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.* **1994**, *46*, 121–136.
10. Emorine, L.J.; Marullo, S.; Briand-Sutren, M.M.; Patey, G.; Tate, K.; Delavier-Klutchko, C.; Strosberg, A.D. Molecular characterization of the human beta 3-adrenergic receptor. *Science* **1989**, *245*, 1118–1121.
11. Harms, H.H.; Zaagsma, J.; Van der Wal, B. Beta-adrenoceptor studies. III. On the beta-adrenoceptors in rat adipose tissue. *Eur. J. Pharmacol.* **1974**, *25*, 87–91.
12. Gauthier, C.; Taverner, G.; Charpentier, F.; Langin, D.; Le Marec, H. Functional beta3-adrenoceptor in the human heart. *J. Clin. Invest.* **1996**, *98*, 556–562.
13. Arioglu-Inan, E.; Kayki-Muthu, G.; Michel, M.C. Cardiac β3-adrenoceptors—A role in human pathophysiology? Br. J. Pharmacol. 2019, 176, 2482–2495.
14. Del Monte, F.; Kaumann, A.J.; Poole-Wilson, P.A.; Wynne, D.G.; Pepper, J.; Harding, S.E. Coexistence of functioning beta 1- and beta 2-adrenoceptors in single myocytes from human ventricle. Circulation 1993, 88, 854–863.
15. Gille, E.; Lemoine, H.; Ehle, B.; Kaumann, A.J. The affinity of (-)-propranolol for beta 1- and beta 2-adrenoceptors of human heart. Differential antagonism of the positive inotropic effects and adenylyl cyclase stimulation by (-)-noradrenaline and (-)-adrenaline. Naunyn. Schmiedebergs. Arch. Pharmacol. 1985, 331, 60–70.
16. Brodde, O.E.; Michel, M.C.; Zerkowski, H.R. Signal transduction mechanisms controlling cardiac contractility and their alterations in chronic heart failure. Cardiovasc. Res. 1995, 30, 570–584.
17. Wachter, S.B.; Gilbert, E.M. Beta-adrenergic receptors, from their discovery and characterization through their manipulation to beneficial clinical application. Cardiology 2012, 122, 104–112.
18. Walsh, D.A.; Van Patten, S.M. Multiple pathway signal transduction by the cAMP-dependent protein kinase. FASEB J. 1994, 8, 1227–1236.
19. Xiao, R.P.; Ji, X.; Lakatta, E.G. Functional coupling of the beta 2-adrenoceptor to a pertussis toxin-sensitive G protein in cardiac myocytes. Mol. Pharmacol. 1995, 47, 322–329.
20. Xiao, R.P.; Avdonin, P.; Zhou, Y.Y.; Cheng, H.; Akhter, S.A.; Eschenhagen, T.; Lefkowitz, R.J.; Koch, W.J.; Lakatta, E.G. Coupling of beta2-adrenoceptor to Gi proteins and its physiological relevance in murine cardiac myocytes. Circ. Res. 1998, 94, 43–52.
21. Strohman, M.J.; Maeda, S.; Hilger, D.; Masureel, M.; Du, Y.; Kobilka, B.K. Local membrane charge regulates beta2 adrenergic receptor coupling to Gβ3. Nat. Commun. 2019, 10, 2234.
22. Cannavo, A.; Koch, W.J. Targeting beta3-Adrenergic Receptors in the Heart: Selective Agonism and beta-Blockade. J. Cardiovasc. Pharmacol. 2017, 69, 71–78.
23. Nantel, F.; Bonin, H.; Emorine, L.J.; Zilberfarb, V.; Strosberg, A.D.; Bouvier, M.; Marullo, S. The human beta-3-adrenoceptor is resistant to short term agonist-promoted desensitization. Mol. Pharmacol. 1993, 43, 548–555.
24. Okeke, K.; Angers, S.; Bouvier, M.; Michel, M.C. Agonist-induced desensitisation of beta3 -adrenoceptors: Where, when, and how? Br. J. Pharmacol. 2019, 176, 2539–2558.
25. Rozec, B.; Gauthier, C. beta3-adrenoceptors in the cardiovascular system: Putative roles in human pathologies. Pharmacol. Ther. 2006, 111, 652–673.
26. Gauthier, C.; Tavernier, G.; Trochu, J.N.; Leblais, V.; Laurent, K.; Langin, D.; Escande, D.; Le Marec, H. Interspecies differences in the cardiac negative inotropic effects of beta(3)-adrenoceptor agonists. J. Pharmaco. Exp. Ther. 1999, 290, 687–693.
27. Gauthier, C.; Leblais, V.; Kobzik, L.; Trochu, J.N.; Khandoudi, N.; Bril, A.; Balligand, J.L.; Le Marec, H. The negative inotropic effect of beta3-adrenoceptor stimulation is mediated by activation of a nitric oxide synthase pathway in human ventricle. J. Clin. Invest. 1998, 102, 1377–1384.
28. Belge, C.; Hammond, J.; Dubois-Deruy, E.; Manoury, B.; Hamelet, J.; Beauloye, C.; Markl, A.; Pouleur, A.C.; Bertrand, L.; Esfahani, H.; et al. Enhanced expression of beta3-adrenoceptors in cardiac myocytes attenuates neurohormone-induced hypertrophic remodeling through nitric oxide synthase. Circulation 2014, 129, 451–462.
29. Moniotte, S.; Kobzik, L.; Feron, O.; Trochu, J.N.; Gauthier, C.; Balligand, J.L. Upregulation of beta(3)-adrenoceptors and altered contractile response to inotropic amines in human failing myocardium. Circulation 2001, 103, 1649–1655.
30. Dal Monte, M.; Evans, B.A.; Arioglu-Inan, E.; Michel, M.C. Upregulation of β 3-adrenoceptors—a general marker of and protective mechanism against hypoxia? Naunyn-Schmiedeberg’s Arch. Pharmacol. 2020, 393, 141–146.
31. Rozec, B.; Noireaud, J.; Trochu, J.N.; Gauthier, C. Place of beta 3-adrenoceptors among other beta-adrenoceptor subtypes in the regulation of the cardiovascular system. Arch. Mal. Coeur. Vaiss. 2003, 96, 905–913.
32. Moniotte, S.; Balligand, J.-L. The β 3-adrenoceptor and its regulation in cardiac tissue. Intensivmedizin und Notfallmedizin 2003, 40, 484–493.
33. Bundgaard, H.; Axelsson, A.; Hartvig, T.J.; Sorgaard, M.; Kofod, K.F.; Hasselbalch, R.; Fry, N.A.; Valeur, N.; Boesgaard, S.; Gustafsson, F.; et al. The first-in-man randomized trial of a beta3 adrenoceptor agonist in chronic heart failure: The BEAT-HF trial. *Eur. J. Heart Fail* 2017, 19, 566–575.

34. Pouleur, A.C.; Anker, S.; Brito, D.; Brosteanu, O.; Hasenclever, D.; Casadei, B.; Edelmann, F.; Filippatos, G.; Gruson, D.; Ikonomidis, I.; et al. Rationale and design of a multicentre, randomized, placebo-controlled trial of mirabegron, a Beta3-adrnergic receptor agonist on left ventricular mass and diastolic function in patients with structural heart disease Beta3-left ventricular hypertrophy (Beta3-LVH). *ESC Heart Fail* 2018, 5, 830–841.

35. Altan, V.M.; Arioglu, E.; Guner, S.; Ozelcikay, A.T. The influence of diabetes on cardiac beta-adrenoceptor subtypes. *Heart Fail. Rev.* 2007, 12, 58–65.

36. Arioglu-Inan, E.; Ozakca, I.; Kayki-Mutlu, G.; Sepici-Dinkel, A.; Altan, V.M. The role of insulin-thyroid hormone interaction on beta-adrenoceptor-mediated cardiac responses. *Eur. J. Pharmacol.* 2013, 718, 533–543.

37. Amour, J.; Loyer, X.; Le Guen, M.; Mabrouk, N.; David, J.S.; Camors, E.; Carusio, N.; Vivien, B.; Andriantsitohaina, R.; Heymes, C.; et al. Altered contractile response due to increased beta3-adrenoceptor stimulation in diabetic cardiomyopathy: The role of nitric oxide synthase 1-derived nitric oxide. *Anesthesiology* 2007, 107, 452–460.

38. Aragno, M.; Mastrocola, R.; Ghe, C.; Arnoletti, E.; Bassino, E.; Alloatti, G.; Muccioli, G. Obestatin induced recovery of myocardial dysfunction in type 1 diabetic rats: Underlying mechanisms. *Cardiovasc. Diabetol.* 2012, 11, 129.

39. Carillion, A.; Feldman, S.; Na, N.; Biais, M.; Carpentier, W.; Birenbaum, A.; Cagnard, N.; Loyer, X.; Bonnefont-Rousselot, D.; Hatem, S.; et al. Atorvastatin reduces beta-Adrenergic dysfunction in rats with diabetic cardiomyopathy. *PloS ONE* 2017, 12, e0180103.

40. Kayki-Mutlu, G.; Arioglu-Inan, E.; Ozakca, I.; Ozelcikay, A.T.; Altan, V.M. beta3-Adrenoceptor-mediated responses in diabetic rat heart. *Gen. Physiol. Biophys.* 2014, 33, 99–109.

41. Okatan, E.N.; Tuncay, E.; Hafez, G.; Turan, B. Profiling of cardiac beta-adrenoceptor subtypes in the cardiac left ventricle of rats with metabolic syndrome: Comparison with streptozotocin-induced diabetic rats. *Can. J. Physiol. Pharmacol.* 2015, 93, 517–525.

42. Dincer, U.D.; Bidasee, K.R.; Guner, S.; Tay, A.; Ozelcikay, A.T.; Altan, V.M. The effect of diabetes on expression of beta1-, beta2-, and beta3-adrenoceptors in rat hearts. *Diabetes* 2001, 50, 455–461.

43. Bidasee, K.R.; Zheng, H.; Shao, C.H.; Parbhoo, S.K.; Rozanski, G.J.; Patel, K.P. Exercise training initiated after the onset of diabetes preserves myocardial function: Effects on expression of beta-adrenoceptors. *J. Appl Physiol* (1985) 2008, 105, 907–914.

44. Saito, K.; Kuroda, A.; Tanaka, H. Characterisation of beta 1 and beta 2 adrenoceptor subtypes in the atrioventricular node of diabetic rat hearts by quantitative autoradiography. *Cardiovasc. Res.* 1991, 25, 950–954.

45. Niclauß, N.; Michel-Reher, M.B.; Alewijnse, A.E.; Michel, M.C. Comparison of three radioligands for the labelling of human β-adrenoceptor subtypes. *Naunyn-Schmiedeberg’s Arch. Pharmacol.* 2006, 374, 99–105.

46. Pradidarcheep, W.; Stallen, J.; Labruyère, W.T.; Dabhoiwala, N.F.; Michel, M.C.; Lamers, W.H. Lack of specificity of commercially available antisera against muscarinic and adrenergic receptors. *Naunyn-Schmiedeberg’s Arch. Pharmacol.* 2009, 379, 397–402.

47. Savarese, J.J.; Berkowitz, B.A. beta-Adrenergic receptor decrease in diabetic rat hearts. *Life Sci.* 1979, 25, 2075–2078.

48. Heyliger, C.E.; Pierce, G.N.; Singal, P.K.; Beamish, R.E.; Dhall, N.S. Cardiac alpha- and beta-adrenergic receptor alterations in diabetic cardiomyopathy. *Basic Res. Cardiol.* 1982, 77, 610–618.

49. Williams, R.S.; Schaible, T.F.; Scheuer, J.; Kennedy, R. Effects of experimental diabetes on adrenergic and cholinergic receptors of rat myocardium. *Diabetes* 1983, 32, 881–886.

50. Ingebretsen, C.G.; Hawelu-Johnson, C.; Ingebretsen, W.R. Jr. Alloxan-induced diabetes reduces beta-adrenergic receptor number without affecting adenylate cyclase in rat ventricular membranes. *J. Cardiovasc. Pharmacol.* 1993, 5, 454–461.

51. Ramanadham, S.; Tenner, T.E. Jr. Alterations in cardiac performance in experimentally-induced diabetes. *Pharmacology* 1983, 27, 130–139.

52. Sylvestre-Gervais, L.; Nadeau, A.; Tancrede, G.; Nuyen, M.; Rousseau-Migneron, S. Decrease in ventricular beta-adrenergic receptors in trained diabetic rats. *Basic Res. Cardiol.* 1984, 79, 432–439.
53. Latifpour, J.; McNeill, J.H. Cardiac autonomic receptors: Effect of long-term experimental diabetes. *J. Pharmacol. Exp. Ther.* 1984, 230, 242–249.
54. Sundaresan, P.R.; Sharma, V.K.; Gingold, S.I.; Banerjee, S.P. Decreased beta-adrenergic receptors in rat heart in streptozotocin-diabetic: Role of thyroid hormones. *Endocrinology* 1984, 114, 1358–1363.
55. Atkins, F.L.; Dowell, R.T.; Love, S. Beta-Adrenergic receptors, adenylate cyclase activity, and cardiac dysfunction in the diabetic rat. *J. Cardiovasc. Pharmacol.* 1985, 7, 66–70.
56. Ramanadham, S.; Tenner, T.E., Jr. Chronic effects of streptozotocin diabetes on myocardial sensitivity in the rat. *Diabetologia* 1986, 29, 741–748.
57. Ramanadham, S.; Young, J.; Tenner, T.E., Jr. Prevention of streptozotocin-induced alterations in the rat heart by 3-O-methyl glucose and insulin treatments. *J. Cardiovasc. Pharmacol.* 1987, 9, 291–297.
58. Ramanadham, S.; Tenner, T.E., Jr. Alterations in the myocardial beta-adrenoceptor system of streptozotocin-diabetic rats. *Eur. J. Pharmacol.* 1987, 136, 377–389.
59. Bitar, M.S.; Koulu, M.; Rapoport, S.I.; Linnoila, M. Adrenal catecholamine metabolism and myocardial adrenergic receptors in streptozotocin diabetic rats. *Biochem. Pharmacol.* 1987, 36, 1011–1016.
60. Nishio, Y.; Kashihwagi, A.; Kida, Y.; Kodama, M.; Abe, N.; Saeki, Y.; Shigeta, Y. Deficiency of cardiac beta-adrenergic receptor in streptozocin-induced diabetic rats. *Diabetes* 1988, 37, 1181–1187.
61. Durante, W.; Sunahara, F.A.; Sen, A.K. Alterations in atrial reactivity in a strain of spontaneously diabetic rats. *Br. J. Pharmacol.* 1989, 97, 1137–1144.
62. Plourde, G.; Martin, M.; Rousseau-Migneron, S.; Nadeau, A. Effect of physical training on ventricular beta-adrenergic receptor adenylate cyclase system of diabetic rats. *Metabolism* 1991, 40, 362–367.
63. Eckel, J.; Gerlach-Eskuchen, E.; Reinauer, H. Alpha-adrenoceptor-mediated increase in cytosolic free calcium in isolated cardiac myocytes. *J. Mol. Cell Cardiol.* 1991, 23, 617–625.
64. Gunasekaran, S.; Young, J.A.; Tenner, T.E., Jr. Pharmacological study of isoproterenol and diabetic cardiomyopathies in rat right ventricular strips. *Pharmacology* 1993, 46, 101–108.
65. Takeda, N.; Dixon, I.M.; Hata, T.; Elinman, V.; Shah, K.R.; Dhalla, N.S. Sequence of alterations in subcellular organelles during the development of heart dysfunction in diabetes. *Diabetes Res. Clin. Pract.* 1996, 30 Suppl., 113–122.
66. Dubois, E.A.; Kam, K.L.; Somsen, G.A.; Boer, G.J.; de Bruin, K.; Batink, H.D.; Pfaffendorf, M.; van Royen, E.A.; van Zwieten, P.A. Cardiac iodine-123 metaiodobenzylguanidine uptake in animals with diabetes mellitus and/or hypertension. *Eur. J. Nucl. Med.* 1996, 23, 901–908.
67. Beenen, O.H.; Batink, H.D.; Pfaffendorf, M.; van Zwieten, P.A. Beta-andrenoceptors in the hearts of diabetic-hypertensive rats: Radioligand binding and functional experiments. *Blood Press.* 1997, 6, 44–51.
68. Matsuda, N.; Hattori, Y.; Gando, S.; Akaishi, Y.; Kanno, M. Diabetes-induced down-regulation of beta1-adrenoceptor mRNA expression in rat heart. *Biochem Pharmacol.* 1999, 58, 881–885.
69. Huissamen, B.; Marais, E.; Genade, S.; Lochner, A. Serial changes in the myocardial beta-adrenergic signaling system in two models of non-insulin dependent diabetes mellitus. *Mol. Cell Biochem.* 2001, 219, 73–82.
70. Bilginoglu, A.; Cicek, F.A.; Ugur, M.; Gurdal, H.; Turan, B. The role of gender differences in beta-adrenergic receptor responsiveness of diabetic rat heart. *Mol. Cell Biochem.* 2007, 305, 63–69.
71. Bilginoglu, A.; Seymen, A.; Tuncay, E.; Zeydanli, E.; Aydemir-Koksoy, A.; Turan, B. Antioxidants but not doxycycline treatments restore depressed beta-adrenergic responses of the heart in diabetic rats. *Cardiovasc. Toxicol.* 2009, 9, 21–29.
72. Gotzsche, O. The adrenergic beta-receptor adenylate cyclase system in heart and lymphocytes from streptozotocin-diabetic rats. In vivo and in vitro evidence for a desensitized myocardial beta-receptor. *Diabetes* 1983, 32, 1110–1116.
73. Sellers, D.J.; Chess-Williams, R. The effect of streptozotocin-induced diabetes on cardiac beta-adrenoceptor subtypes in the rat. *J. Auton. Pharmacol.* 2001, 21, 15–21.
74. Mooradian, A.D.; Morley, J.E.; Scarpace, P.J. The role of zinc status in altered cardiac adenylyl cyclase activity in diabetic rats. *Acta Endocrinol. (Copenh).* 1988, 119, 174–180.
75. Tuncay, E.; Okatan, E.N.; Vassort, G.; Turan, B. ss-blocker timolol prevents arrhythmogenic Ca(2)(+) release and normalizes Ca(2)(+) and Zn(2)(+) dyshomeostasis in hyperglycemic rat heart. *PLoS ONE* 2013, 8, e71014.
76. Cros, G.; Chanez, P.; Michel, A.; McNeill, J.; Serrano, J. Cardiac beta-adrenergic receptors in diabetic rats: Alteration of guanyl nucleotide regulation. *J. Pharmacol.* 1986, 17, 595–600.
77. Gotzsche, L.B.; Rosenqvist, N.; Gronbaek, H.; Flyvbjerg, A.; Gotzsche, O. Increased number of myocardial voltage-gated Ca\textsuperscript{2+} channels and unchanged total beta-receptor number in long-term streptozotocin-diabetic rats. *Eur. J. Endocrinol.* 1996, 134, 107–113.

78. Myers, R.B.; Fomovsky, G.M.; Lee, S.; Tan, M.; Wang, B.F.; Patwari, P.; Yoshioka, J. Deletion of thioerodixin-interacting protein improves cardiac inotropic reserve in the streptozotocin-induced diabetic heart. *Am. J. Physiol. Heart Circ. Physiol.* 2016, 310, H1748–H1759.

79. Austin, C.E.; Chess-Williams, R. Transient elevation of cardiac beta-adrenoceptor responsiveness and receptor number in the streptozotocin-diabetic rat. *J. Auton Pharmacol.* 1992, 12, 205–214.

80. Austin, C.E.; Chess-Williams, R. Diabetes-induced changes in cardiac beta-adrenoceptor responsiveness: Effects of aldose reductase inhibition with ponalrestat. *Br. J. Pharmacol.* 1991, 102, 478–482.

81. Roth, D.A.; White, C.D.; Hamilton, C.D.; Hall, J.L.; Stanley, W.C. Adrenergic desensitization in left ventricle from streptozotocin diabetic swine. *J. Mol. Cell. Cardiol.* 1995, 27, 2315–2325.

82. Stanley, W.C.; Dore, J.J.; Hall, J.L.; Hamilton, C.D.; Pizzurro, R.D.; Roth, D.A. Diabetes reduces right atrial beta-adrenergic signaling but not agonist stimulation of heart rate in swine. *Can. J. Physiol. Pharmacol.* 2001, 79, 346–351.

83. Zola, B.E.; Miller, B.; Stiles, G.L.; Sonnenblick, E.H.; Fein, F.S. Heart rate control in diabetic rabbits: Blunted response to isoproterenol. *Am. J. Physiol.-Endocrinol. Metab.* 1988, 255, E636–E641.

84. Lee, J.R.; Zhang, X.-J.; Lin, B.-K.; Reigel, C.E.; Tenner, J.; Thomas, E. Altered inotropic reactivity in diabetic rabbit right ventricular myocardium. *Can. J. Physiol. Pharmacol.* 2004, 82, 903–910.

85. Uekita, K.; Tobise, K.; Onodera, S. Enhancement of the cardiac \(\beta\)-adrenergic system at an early diabetic state in spontaneously diabetic Chinese hamsters. *Jpn. Circulation J.* 1997, 61, 64–73.

86. Amour, J.; Loyer, X.; Michelet, P.; Birenbaum, A.; Riou, B.; Heymes, C. Preservation of the positive lusitropic effect of beta-adrenoceptors stimulation in diabetic cardiomyopathy. *Anesth. Analg.* 2008, 107, 1130–1138.

87. Sharma, V.; Parsons, H.; Allard, M.F.; McNeill, J.H. Metoprolol increases the expression of beta(3)-adrenoceptors in the diabetic heart: Effects on nitric oxide signaling and forkhead transcription factor-3. *Eur. J. Pharmacol.* 2008, 595, 44–51.

88. Lahaye Sle, D.; Gratas-Delamarache, A.; Malarde, L.; Vincent, S.; Zguira, M.S.; Morel, S.L.; Delamarche, P.; Zouhal, H.; Carre, F.; Bekono, F.R. Intense exercise training induces adaptation in expression and responsiveness of cardiac beta-adrenoceptors in diabetic rats. *Cardiovasc. Diabetol.* 2010, 9, 72.

89. Mishra, P.K.; Givvimani, S.; Metreveli, N.; Tyagi, S.C. Attenuation of beta2-adrenergic receptors and homocysteine metabolic enzymes cause diabetic cardiomyopathy. *Biochem. Biophys. Res. Commun.* 2010, 401, 175–181.

90. Le Douairon Lahaye, S.; Rebillard, A.; Zguira, M.S.; Malarde, L.; Saiag, B.; Gratas-Delamarache, A.; Carre, F.; Bekono, F.R. Effects of exercise training combined with insulin treatment on cardiac NOS1 signaling pathways in type 1 diabetic rats. *Mol. Cell Biochem.* 2011, 347, 53–62.

91. Monnerat-Cahil, G.; Trentin-Sonoda, M.; Guerra, B.; Manso, G.; Ferreira, A.C.; Silva, D.L.; Coutinho, D.C.; Carneiro-Ramos, M.S.; Rodrigues, D.C.; Cabral-da-Silva, M.C.; et al. Bone marrow mesenchymal stromal cells rescue cardiac function in streptozotocin-induced diabetic rats. *Int. J. Cardiol.* 2014, 171, 199–208.

92. Garris, D.R. The effects of estradiol and progesterone on reproductive tract atrophy and tissue adrenergic indices in diabetic C57BL/KsJ mice. *Proc. Soc. Exp. Biol Med.* 1990, 193, 39–45.

93. Schaffer, S.; Allo, S.; Punna, S.; White, T. Defective response to cAMP-dependent protein kinase in non-insulin-dependent diabetic heart. *Am. J. Physiol.-Endocrinol. Metab.* 1991, 261, E369–E376.

94. Lambert, R.R.; Lingam, S.J.; Wang, H.-Y.; Bollen, I.A.; Hughes, G.; Galvin, I.F.; Bunton, R.W.; Bahn, A.; Katar, R.; Baldi, J.C. Impaired relaxation despite upregulated calcium-handling protein atrial myocardium from type 2 diabetic patients with preserved ejection fraction. *Cardiovasc. Diabetol.* 2014, 13, 72.

95. Jiang, J.; Li, N.; Wang, X.; Lu, Y.; Bi, Y.; Wang, W.; Li, X.; Ning, G. Aberrant expression and modification of silencing mediator of retinoic acid and thyroid hormone receptors involved in the pathogenesis of tumoral cortisol resistance. *Endocrinology* 2010, 151, 3697–3705.

96. Fu, Q.; Hu, Y.; Wang, Q.; Liu, Y.; Li, N.; Xu, B.; Kim, S.; Chiamvimonvat, N.; Xiang, Y.K. High–fat diet induces protein kinase A and G–protein receptor kinase phosphorylation of \(\beta2\)-adrenergic receptor and impairs cardiac adrenergic reserve in animal hearts. *J. Physiol.* 2017, 595, 1973–1986.
Diabetes alters the myocardium. Exercise does not activate the β 3 adrenergic receptor-adenyl cyclase, cyclic AMP phosphodiesterase and eNOS. Phosphodiesterase 5 associates with β 3-adrenoceptors at both the onset and after sustained hyperglycemia in diabetic rats. 

97. Thackeray, J.T.; Parsa-Nezhad, M.; Kenk, M.; Thorn, S.L.; Kolajova, M.; Beanlands, R.S.; DaSilva, J.N. Reduced CGP12177 binding to cardiac beta-adrenoceptors in hyperglycemic high-fat-diet-fed, streptozotocin-induced diabetic rats. *Nacl. Med. Biol.* 2011, 38, 1059–1066.

98. Kleindienst, A.; Battault, S.; Belaidi, E.; Tanguy, S.; Rosselin, M.; Boulhobra, D.; Meyer, G.; Gayrard, S.; Walther, G.; Geny, B. Exercise does not activate the β 3 adrenergic receptor–eNOS pathway, but reduces inducible NOS expression to protect the heart of obese diabetic mice. *Basic Res. Cardiol.* 2016, 111, 40.

99. Jiang, C.; Carillion, A.; Na, N.; De Jong, A.; Feldman, S.; Lacorte, J.-M.; Bonnefont-Rousselot, D.; Riou, B.; Amour, J. Modification of the β-adrenoceptor stimulation pathway in Zucker obese and obese diabetic rat myocardium. *Crit. Care Med.* 2015, 43, E241–E249.

100. Haley, J.M.; Thackeray, J.T.; Kolajova, M.; Thorn, S.L.; DaSilva, J.N. Insulin therapy normalizes reduced myocardial beta-adrenoceptors at both the onset and after sustained hyperglycemia in diabetic rats. *Life Sci.* 2015, 132, 101–107.

101. Haley, J.M.; Thackeray, J.T.; Thorn, S.L.; DaSilva, J.N. Cardiac beta-Adrenoceptor Expression Is Reduced in Zucker Diabetic Fatty Rats as Type-2 Diabetes Progresses. *PLoS ONE* 2015, 10, E0127581.

102. Thaung, H.P.; Baldi, J.C.; Wang, H.Y.; Hughes, G.; Cook, R.F.; Bussey, C.T.; Sheard, P.W.; Bahn, A.; Jones, P.P.; Schwenke, D.O.; et al. Increased Efferent Cardiac Sympathetic Nerve Activity and Defective Intrinsic Heart Rate Regulation in Type 2 Diabetes. *Diabetes* 2015, 64, 2944–2956.

103. Dincer, U.D.; Guner, S.; Tay, A.; Arioglu, E.; Tasdelen, A.; Aslamaci, S.; Bidasee, K.R. Decreased expression of beta1- and beta2-adrenoceptors in human diabetic atrial appendage. *Cardiovasc. Diabetol.* 2003, 2, 6.

104. Daniels, A.; Van Bilsen, M.; Janssen, B.; Brouns, A.; Cleutjens, J.; Roemen, T.; Schaart, G.; Van Der Hage, A.N.; Herman, E.H.; Jordan, A.W.; Ferrans, V.J. Adrenergic modulation of phosphodiesterase 5 Associates With β 3-adrenoceptors in human diabetic atrial appendage. *Cell Metab.* 2014, 12, 29250–29258.

105. Srivastava, A.K.; Anand-Srivastava, M.B. Streptozotocin-induced diabetes and hormone sensitivity of adenyl cyclase in rat myocardial sarclemma, aorta and liver. *Biochem. Pharmacol.* 1985, 34, 2013–2017.

106. Ingebretsen Jr, W.; Peralta, C.; Monscher, M.; Wagner, L.; Ingebretsen, C. Diabetes alters the myocardial cAMP-protein kinase cascade system. *Am. J. Physiol.-Heart Circulatory Physiol.* 1981, 240, H375–H382.

107. Miller Jr, T. Phosphorylase activation hypersensitivity in hearts of diabetic rats. *Am. J. Physiol.-Endocrinol. Metab.* 1984, 246, E134–E140.

108. Vadlamudi, R.; McNEILL, J.H. Effect of experimental diabetes on rat cardiac cAMP, phosphorylase, and inotropy. *Am. J. Physiol.-Heart Circulatory Physiol.* 1983, 244, H844–H851.

109. Das, I. Effect of diabetes and insulin on the rat heart adenyl cyclase, cyclic AMP phosphodiesterase and cyclic AMP. *Hormone Metab. Res.* 1973, 5, 330–333.

110. Bockus, L.B.; Humphries, K.M. cAMP-dependent protein kinase (PKA) signaling is impaired in the diabetic heart. *J. Biol. Chem.* 2015, 290, 29250–29258.

111. El-Hage, A.N.; Herman, E.H.; Jordan, A.W.; Ferrans, V.J. Influence of the diabetic state on isoproterenol-induced cardiac necrosis. *J. Mol. Cell. Cardiol.* 1985, 17, 361–369.

112. West, T.M.; Wang, Q.; Deng, B.; Zhang, Y.; Barbagallo, F.; Reddy, G.R.; Chen, D.; Phan, K.S.; Xu, B.; Isidori, A. Phosphodiesterase 5 Associates With β2 Adrenergic Receptor to Modulate Cardiac Function in Type 2 Diabetic Hearts. *J. Am. Heart Assoc.* 2019, 8, E012273.

113. Hilsted, J.; Richter, E.; Madsbad, S.; Tronier, B.; Christensen, N.J.; Hildebrandt, P.; Damkjaer, M.; Galbo, H. Metabolic and cardiovascular responses to epinephrine in diabetic neuropathy. *N. Engl. J. Med.* 1987, 317, 421–426.

114. Trovik, T.; Jaeger, R.; Jorde, R.; Sager, G. Reduced sensitivity to beta--adrenoceptor stimulation and blockade in insulin dependent diabetic patients with hypoglycaemia unawareness. *Br. J. Clin. Pharmacol.* 1994, 38, 427–432.

115. Wang, Q.; Liu, Y.; Fu, Q.; Xu, B.; Zhang, Y.; Kim, S.; Tan, R.; Barbagallo, F.; West, T.; Anderson, E. Inhibiting insulin-mediated β2-adrenergic receptor activation prevents diabetes-associated cardiac dysfunction. *Circulation* 2017, 135, 73–88.

116. Wichelhaus, A.; Russ, M.; Petersen, S.; Eckel, J.G. protein expression and adenylyl cyclase regulation in ventricular cardiomyocytes from STZ-diabetic rats. *Am. J. Physiol.-Heart Circulatory Physiol.* 1994, 267, H548–H555.

117. Smith, C.; Pierce, G.; Dhall, N. Alterations in adenylyl cyclase activity due to streptozotocin-induced diabetic cardiomyopathy. *Life Sci.* 1984, 34, 1223–1230.
163. Foy, J.; Lucas, P. Effect of experimental diabetes, food deprivation and genetic obesity on the sensitivity of pithed rats to autonomic agents. *Br. J. Pharmacol.* **1976**, *57*, 229.

164. Paulson, D.J.; Kopp, S.J.; Tow, J.P.; Feliksik, J.M.; Peace, D.G. Impaired in vivo myocardial reactivity to norepinephrine in diabetic rats. *Proc. Soc. Exp. Biol. Med.* **1986**, *183*, 186–192.

165. Hoit, B.D.; Castro, C.; Bullton, G.; Knight, S.; Mathil, M.A. Noninvasive evaluation of cardiac dysfunction by echocardiography in streptozotocin-induced diabetic rats. *J. Cardiac Fail.* **1999**, *5*, 324–333.

166. Irlbeck, M.; Zimmer, H. The functional and metabolic responses of the heart to catecholamines are attenuated in diabetic rats. *Cardioscience* **1995**, *6*, 131–138.

167. Irlbeck, M.; Zimmer, H.G. Functional responses of the left and right heart of diabetic rats to α- and β-adrenergic receptor stimulation. *Diabetes Res. Clin. Pract.* **1996**, *31*, S79–S86.

168. Borges, G.R.; De Oliveira, M.; Salgado, H.C.; Fazan, R. Myocardial performance in conscious streptozotocin diabetic rats. *Cardiovascular Diabetology* **2006**, *5*, 26.

169. Hall, J.L.; Stanley, W.C.; Lopaschuk, G.D.; Wisneski, J.A.; Pizzurro, R.D.; Hamilton, C.D.; McCormack, J.G. Impaired pyruvate oxidation but normal glucose uptake in diabetic pig heart during dobutamine-induced work. *Am. J. Physiol.-Heart Circulatory Physiol.* **1996**, *271*, H1320–H1329.

170. Berlin, I.; Grimaldi, A.; Bosquet, F.; Puech, A.J. Decreased β-adrenergic sensitivity in insulin-dependent diabetic subjects. *J. Clin. Endocrinol. Metab.* **1986**, *63*, 262–265.

171. Fritsche, A.; Stumvoll, M.; Grub, M.; Sieslack, S.; Renn, W.; Schmülling, R.-M.; Häring, H.-U.; Gerich, J.E. Effect of hypoglycemia on β-adrenergic sensitivity in normal and type 1 diabetic subjects. *Diabetes Care* **1998**, *21*, 1505–1510.

172. Korytkowski, M.T.; Mokan, M.; Veneman, T.F.; Mitrakou, A.; Cryer, P.E.; Gerich, J.E. Reduced β-adrenergic sensitivity in patients with type 1 diabetes and hypoglycemia unawareness. *Diabetes Care* **1998**, *21*, 1939–1943.

173. Dejgaard, A.; Andersen, P.; Hvidberg, A.; Hilsted, J. Cardiovascular, metabolic, and hormonal responses to noradrenaline in diabetic patients with autonomic neuropathy. *Diabetic Med.* **1996**, *13*, 983–989.

174. Steinhorn, B.; Sartoretto, J.L.; Sorrentino, A.; Romero, N.; Kalwa, H.; Abel, E.D.; Michel, T. Insulin-dependent metabolic and inotropic responses in the heart are modulated by hydrogen peroxide from NAPDH-oxidase isoforms NOX2 and NOX4. *Free Radical Biol. Med.* **2017**, *113*, 16–25.

175. Rodriguez, G.; Mago, N. Inotropic and chronotropic effects of propranolol in isolated atrium of rats with fructose-induced insulin-resistance. *Investigacion Clin.* **2017**, *58*, 22–33.

176. Cook, R.F.; Bussey, C.T.; Fomison-Nurse, I.C.; Hughes, G.; Bahn, A.; Cragg, P.A.; Lamberts, R.R. Beta2-Adrenoceptors indirectly support impaired beta1-adrenoceptor responsiveness in the isolated type 2 diabetic rat heart. *Exp. Physiol.* **2019**, *104*, 808–818.

177. Takada, A.; Miki, T.; Kuno, A.; Kouzu, H.; Sunaga, D.; Itoh, T.; Tanno, M.; Yano, T.; Sato, T.; Ishikawa, S. Role of ER stress in ventricular contractile dysfunction in type 2 diabetes. *PLoS ONE* **2012**, *7*, E39893.

178. Wilson, G.A.; Wilson, L.C.; Lamberts, R.R.; Majeed, K.; Lal, S.; Wilkins, G.T.; Baldi, J.C. β-Adrenergic Responsiveness in the Type 2 Diabetic Heart: Effects on Cardiac Reserve. *Med. Sci. Sports Exercise* **2017**, *49*, 907–914.

179. Bussey, C.T.; de Leeuw, A.E.; Lamberts, R.R. Increased haemodynamic adrenergic load with isoflurane anaesthesia in type 2 diabetic and obese rats in vivo. *Cardiovasc. Diabetol.* **2014**, *13*, 161.

180. Cook, R.F.; Bussey, C.T.; Mellor, K.M.; Cragg, P.A.; Lamberts, R.R. beta1 -Adrenoceptor, but not beta2-adrenoceptor, subtype regulates heart rate in type 2 diabetic rats in vivo. *Exp. Physiol.* **2017**, *102*, 911–923.

181. Gregg, E.W.; Li, Y.; Wang, J.; Rios Burrows, N.; Ali, M.K.; Rolka, D.; Williams, D.E.; Geiss, L. Changes in diabetes-related complications in the United States, 1990–2010. *N. Engl. J. Med.* **2014**, *370*, 1514–1523.

182. Cheng, H.-J.; Zhang, Z.-S.; Onishi, K.; Ukai, T.; Sane, D.C.; Cheng, C.P. Upregulation of functional β3-adrenergic receptor in the failing canine myocardium. *Circulation Res.* **2001**, *89*, 599–606.

183. García-Prieto, J.; García-Ruiz, J.M.; Sanz-Rosa, D.; Pun, A.; García-Alvarez, A.; Davidson, S.M.; Fernández-Friera, L.; Nuno-Ayala, M.; Fernández-Jiménez, R.; Bernal, J.A. β3 adrenergic receptor selective stimulation during ischemia/reperfusion improves cardiac function in translational models through inhibition of mPTP opening in cardiomyocytes. *Basic Res. Cardiol.* **2014**, *109*, 422.
184. Aragón, J.P.; Condit, M.E.; Bhushan, S.; Predmore, B.L.; Patel, S.S.; Grinsfelder, D.B.; Gundewar, S.; Jha, S.; Calvert, J.W.; Barouch, L.A. Beta3-adrenoreceptor stimulation ameliorates myocardial ischemia-reperfusion injury via endothelial nitric oxide synthase and neuronal nitric oxide synthase activation. *J. Am. Coll. Cardiol.* 2011, 58, 2683–2691.

185. Goyal, R.; Jialal, I. Diabetes Mellitus Type 2. In: StatPearls StatPearls Publishing, Treasure Island (FL). 2019.

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