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Abnormal cannabidiol confers cardioprotection in diabetic rats independent of glycemic control

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Abstract

Chronic GPR18 activation by its agonist abnormal cannabidiol (trans-4-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol; abn-cbd) improves myocardial redox status and function in healthy rats. Here, we investigated the ability of abn-cbd to alleviate diabetes-evoked cardiovascular pathology and the contribution of GPR18 to this effect. Four weeks after diabetes induction by streptozotocin (STZ, 55 mg/kg; i.p), male Wistar rats received abn-cbd, the GPR18 antagonist (1,3-dimethoxy-5-methyl-2-[(1R,6R)-3-methyl-6-(1-methylethenyl)-2-,cyclohexen-1-yl]benzene;O-1918), their combination (100 μg/kg/day, i.p, each) or their vehicle for 2 weeks. Abn-cbd had no effect on diabetes-evoked cardiac hypertrophy or impaired glycemic control (hyperglycemia and hypoinsulinemia), but alleviated the associated reductions in left ventricular (LV) contractility (dP/dt_{max}) and relaxation (dP/dt_{min}) indices, and the increases in LV end diastolic pressure (LVEDP) and cardiac vagal dominance. Abn-cbd also reversed myocardial oxidative stress by restoring circulating and cardiac nitric oxide (NO) and adiponectin (ADN) levels and enhancing GPR18 expression and phosphorylation of Akt, ERK1/2 and eNOS in diabetic rats' hearts. Concurrent GPR18 blockade (O-1918) abrogated all favorable effects of abn-cbd in diabetic rats. Collectively, the current findings present evidence for abn-cbd alleviation of diabetes-evoked cardiovascular anomalies likely via GPR18 dependent restoration of cardiac adiponectin-Akt-eNOS signaling and the diminution of myocardial oxidative stress.

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Keywords

diabetes; GPR-18; abnormal cannabidiol; O-1918; cardiac dysfunction; adiponectin

1. Introduction

Diabetes causes LV dysfunction and cardiac autonomic abnormalities (Vinik and Ziegler, 2007) via several mechanisms including: (i) hyperglycemia-induced overproduction of reactive oxygen species (Brownlee, 2001), (ii) impaired antioxidant enzyme activities (Bukan et al., 2004), and (iii) reductions in ADN (Guo et al., 2007) and cardiac cell survival molecules such as Akt (Van Linthout et al., 2008) levels.

While a cardioprotective role for endocannabinoids (ECs) might seem unreasonable because current knowledge implicates ECs in diabetes-induced inflammation, oxidative stress, and cardiovascular dysfunction (Horvath et al., 2012), we must consider other important findings. First, the detrimental cardiovascular effects of ECs are mostly mediated by the cannabinoid receptor 1, CB1R (Ibrahim and Abdel-Rahman, 2015; Rajesh et al., 2012; Varga et al., 1995). Second, activation of the novel EC receptor, GPR18, by its endogenous (N-arachidonylglycine; NAGly) (Burstein et al., 2011; Kohno et al., 2006) or synthetic (trans-4-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol; abn-cbd) ligand causes hypotension (Offertaler et al., 2003; Parmar and Ho, 2010). Importantly, these latter studies were conducted in anesthetized animals following acute abn-cbd administration, and did not provide any insight into the cardiac effects or the mechanism of GPR18-mediated hypotension (Johns et al., 2007; Offertaler et al., 2003).

GPR18 exhibits highest expression in spleen and testis followed by thymus gland, small intestine and peripheral blood leukocytes and is not expressed in the liver (Gantz et al., 1997; Kohno et al., 2006; Penumarti and Abdel-Rahman, 2014b). Our recent study is the first to show GPR18 expression in the heart, and that chronic abn-cbd administration reduces blood pressure (BP) and improves LV function in healthy rats (Matouk et al., 2017). These abn-cbd evoked cardiovascular effects are likely GPR18-mediated because concurrent administration of the GPR18 antagonist (1,3-dimethoxy-5-methyl-2-[(1R,6R)-3-methyl-6-(1-methylethenyl)-2-,cyclohexen-1-yl]benzene; O-1918), in the same dose as abn-cbd, abrogated these responses (Matouk et al., 2017; Offertaler et al., 2003; Penumarti and Abdel-Rahman, 2014b). While still debated (Pertwee et al., 2010; Ross, 2009), reported findings ruled out the involvement of other EC receptors such as GPR55 in the abn-cbd mediated cardiovascular effects (Caldwell et al., 2013; Johns et al., 2007).

A plausible role for ADN in GPR18-mediated cardiovascular effects (Matouk et al., 2017; Penumarti and Abdel-Rahman, 2014a; b) gains credence from ADN ability to reduce reactive oxygen species by preserving pro-survival signaling molecules such as Akt and ERK1/2, and by increasing NOS-derived NO levels (Margaritis et al., 2013; Tao et al., 2007). Importantly, whether a reduction in circulating ADN levels in diabetes (Guo et al., 2007) contributes to diabetes associated cardiovascular anomalies remains controversial (Witberg et al., 2016).

The current knowledge on the abn-cbd evoked hypotension and salutary cardiac effects is based on findings in healthy animals. Therefore, our main goal was to test the hypothesis that abn-cbd alleviates cardiovascular pathology in diabetes, at least partly, via GPR18-dependent restoration of the ADN-eNOS/NO cascade. We also determined if reversal of metabolic maladaptations (hyperglycemia and hypoinsulinemia) or the associated cardiac hypertrophy accounts for the abn-cbd evoked favorable cardiovascular effects in diabetic rats. To achieve these goals, four weeks after STZ-induced diabetes, the rats received abn-cbd, O-1918, their combination or vehicle daily for 2 weeks, and non-diabetic rats served as controls. Hemodynamic measurements and oral glucose tolerance test were conducted in conscious rats before collecting blood and cardiac tissues for ex vivo biochemical studies.

2. Materials and methods

2.1. Preparation of the rats

Male Wistar rats (250–300 g; Charles River Laboratories, Raleigh, NC) were used in the present study. The rats were housed two per cage in a room with a controlled environment at a constant temperature of 23° ± 1°C, 50 ± 10% humidity and a 12 h light/dark cycle. Food (Prolab Rodent Chow, Prolab RMH 3000; Granville Milling, Creedmoor, NC) and water were provided ad libitum. All surgical, experimental and animal care procedures were conducted in accordance with, and approved by, the East Carolina University Institutional Animal Care and Use Committee and in accordance with the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 2011).

2.2. Induction of diabetes

After the acclimatization period, rats were fasted overnight before induction of diabetes by a single STZ dose (55 mg/kg, i.p, Sigma Co., St. Louis, MO, USA) freshly dissolved in 0.1 M citrate buffer, pH 4.0. The rats were allowed to drink 5% dextrose solution overnight. STZ causes irreversible necrosis of insulin producing pancreatic β-cells leading to the development of diabetes and associated abnormalities such as polyuria, polydipsia and elevated blood glucose (BGL) level (Schnedl et al., 1994). After 2 days, diabetes was confirmed by the presence of BGL > 300 mg/dl, measured in tail vein blood (ReliOn®, Prime Blood Glucose Meter, USA). Buffer-treated rats were used as non-diabetic controls as reported in our previous study (El-Sayed et al., 2016).

2.3. Protocol and experimental groups

Four weeks after STZ injection, 4 groups of diabetic rats (n = 8, each) received one of the following daily intraperitoneal (i.p) injections for 2 weeks: (i) the vehicle for abn-cbd/O-1918 (DMSO: PBS 1:3, pH 7.2), (ii) abn-cbd (100 μg/kg), (iii) O-1918 (100 μg/kg) or (iv) O-1918 followed 30 min later with abn-cbd (100 μg/kg, each). The abn-cbd and O-1918 doses and their vehicle were based on our previous study (Matouk et al., 2017), and preliminary findings (Fig. 1S). An additional group of non-diabetic rats, served as control, received citrate buffer (vehicle for STZ) and the vehicle for abn-cbd/O-1918. At the conclusion of the 2-week treatment period, hemodynamic measurements in conscious rats and ex vivo biochemical analyses were conducted as described in the following sections.

2.4. Oral glucose tolerance test (OGTT)

Two days before the conclusion of the study, animals were fasted overnight, and subjected to the OGTT by measuring BGL (ReliOn®, Prime Blood Glucose Meter, USA) at 0, 30, 60, 90 and 120 min after oral administration of 10% glucose solution (1 g/10 ml/kg) as reported (Frankenfeld et al., 2014).

2.5. Hemodynamic measurements

At the end of the treatment period, arterial and LV catheterizations were conducted for measurements of mean arterial pressure (MAP) and LV function, respectively. Surgical procedures were performed under sterile conditions and anesthesia using ketamine (9 mg/100 g) and xylazine (1 mg/100 g, i.p.); pre- and post-surgical analgesia (buprenorphine; 0.03 mg/kg, s.c, Reckitt Benckiser, Richmond, VA) in accordance with IACUC guidelines. Thereafter, the rats were housed individually, and allowed 24 h to recover from anesthesia. On the day of the experiment, the catheters were connected to Gould-Statham pressure transducers (Gould Inc., Oxnard, CA) for hemodynamic measurements in conscious unrestrained rats as in our previous studies (El-Sayed et al., 2016; Matouk et al., 2017). The measured LV function indices included dP/dt_{max} (the maximum rate of isovolumic pressure development, an index of myocardial contractility), dP/dt_{min} (the maximum rate of isovolumic pressure decline, an index of myocardial relaxation) and the left ventricular end diastolic pressure (LVEDP; an index of ventricular performance and intravascular pressure and volume). MAP was computed as [1/3 (systolic pressure-diastolic pressure) + diastolic pressure] and heart rate (HR) was extracted from BP values. BP and HR were allowed to stabilize at least 30 min before collecting and analyzing hemodynamic data over 1 h using ML870 (Power Lab 8/30) and Lab Chart 7 software (AD Instruments, Colorado Spring, CO) as in our previous studies (El-Sayed et al., 2016; Matouk et al., 2017).

2.6. Frequency domain analysis

Frequency domain analysis was conducted to determine the relative cardiac sympathetic and parasympathetic dominance by using software designed for rats (Nevrokard SA-BRS; Izola, Slovenia). The power of RR interval (RRI) spectral density oscillations was computed by 512- point fast Fourier transform and integrated over the specified low-frequency (LF, 0.25–0.75 Hz) to high-frequency (HF, 0.75–5.0 Hz) range (Shaltout and Abdel-Rahman, 2005). LF_{RRI} and HF_{RRI} values reflect sympathetic and parasympathetic dominance, respectively, whereas the $LF/HF_{(RRI)}$ ratio is a measure of sympathovagal balance. However, some studies indicate that LF represents the changes in both sympathetic and parasympathetic activities (Appel et al., 1989) because the calculation of LF_{RRI} and HF_{RRI} in the absolute units allows the change in the total power to affect the LF and HF in the same direction. Therefore, the calculation of their values in normalized units (n.u), followed in this study, minimizes this effect (Malliani et al., 1991).

2.7. Body and heart weights and tissue collection

Determination of the body weights was done on the first day of STZ or vehicle administration and weekly till the end of the study. At the end of the hemodynamic measurements, blood was collected, centrifuged, and stored at −80°C until use. Then

animals were euthanized by injecting a lethal dose of sodium pentobarbital (100 mg/kg) and the hearts were removed, cleaned and weighed to determine the heart weight (HW)/body weight (BW) ratio (an index of myocardial hypertrophy) as in our previous study (El-Sayed et al., 2016). Hearts was then flash-frozen in 2- methylbutane on dry ice, and stored at −80°C until use.

2.8. Western blot analysis

These analyses were conducted in accordance with our recent study (Matouk et al., 2017). Heart tissues were homogenized on ice in lysis buffer (pH 7.5) containing 20 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerolphosphate, 1 mM activated sodium orthovanadate, 1 μg/ml leupeptin, and protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). Samples were centrifuged at 4°C then protein content was determined in the supernatant (Bio-Rad Laboratories, Hercules, CA). Proteins in the sample were separated by gel electrophoresis using sodium dodecyl sulfate polyacrylamide gel (NuPAGE 4%–12% Bis-Tris Gel; Invitrogen, Carlsbad, CA) and MOPS NuPAGE running buffer. Proteins were transferred from the gels to nitrocellulose membranes using TransBlot SD transfer cell (Bio-Rad Laboratories, Hercules, CA). The membranes were incubated at room temperature for 2 h in Odyssey Blocking Buffer (LI-COR Biosciences, Lincoln, NE), and were then incubated at 4°C overnight in a mixture of: (i) mouse monoclonal anti-GAPDH (1:15000; Abcam Inc., Cambridge, MA) with rabbit polyclonal anti-GPR18 (1:500; assay biotech., Sunnyvale, CA) or anti-adiponectin receptor-1 (AdipoR1) or anti-adiponectin receptor-2 (AdipoR2) (1:200 each; Santa Cruz Biotechnology Inc., CA) or anti-ADN (1:200 Abcam Inc., Cambridge, MA); (ii) mouse monoclonal anti-beta-actin (1:15000; Abcam Inc., Cambridge, MA) with rabbit polyclonal anti-iNOS (1:200; BD Biosciences, San Jose, CA); (iii) rabbit polyclonal anti-eNOS with mouse monoclonal antiphospho-eNOS (pSer1177) (1:200 each; BD Biosciences, San Jose, CA); (iv) rabbit polyclonal anti-ERK1/2 (1:500) with mouse monoclonal antiphospho-ERK1/2 (pTyr202/Y204) (1:500); and (v) rabbit polyclonal anti-Akt (1:500) with mouse monoclonal antiphospho-Akt (pSer473) (1:500). Antibodies for Akt, p-Akt, ERK1/2, and p-ERK1/2 were obtained from (Cell Signaling Technology, Inc. Danvers, MA). Each antibody was diluted in Odyssey Blocking Buffer and 0.1% tween. At the end of the incubation, membranes were washed with PBS containing 0.1% tween-20 (PBS-T) and incubated for 1h with fluorescently labeled secondary antibody mixture containing IRDye 680-conjugated goat anti-mouse and IRDye 800-conjugated goat anti-rabbit (1:15000; LI-COR Biosciences., Lincoln, NE). Bands were detected by using Odyssey Infrared Imager and analyzed with Odyssey application software (LI-COR Biosciences). GPR18, AdipoR1, AdipoR2 and ADN expressions were quantified against GAPDH, whereas iNOS expression was quantified against beta-actin and p-eNOS, p-ERK1/2 and p-Akt were quantified against the corresponding total proteins. Western blot and enzymatic activity data for each protein were expressed as percent of control (vehicle-treated rats) as in our previous studies (Matouk et al., 2017; Penumarti and Abdel-Rahman, 2014b). Validation of the antibody specificity was conducted by omitting the primary or secondary antibody, the use of specific blocking peptide and/or the use of positive and negative controls. The latter validation was used for validating the GPR18 antibody in GPR18 rich (spleen) or deprived (liver) tissues in our previous studies (Matouk et al., 2017; Penumarti and Abdel-Rahman, 2014b).

2.9. Measurement of nitrate/nitrite (NOx)

Serum and myocardial nitrate/nitrite levels (index of NO) were measured colorimetrically by commercially available kit according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI) and our previous studies (Matouk et al., 2017; Penumarti and Abdel-Rahman, 2014b).

2.10. Measurement of plasma adiponectin and insulin levels

Rat Adiponectin ELISA kit (B-Bridge International Inc., San Jose, CA) and Rat Insulin ELISA kit (EMD Millipore Corp., Charles, MO) were used for measurement of plasma ADN and insulin levels according to the manufacturer's instructions and reported studies (Matouk et al., 2017; Nakamaki et al., 2011; Pae and Kim, 2014).

2.11. Measurement of myocardial antioxidant catalase activity

Catalase activity was determined in heart homogenates colorimetrically using the Catalase Assay Kit (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions and our previous study (Ibrahim et al., 2014).

2.12. Measurement of myocardial oxidative stress (expressed as Malondialdehyde "MDA"Levels)

Malondialdehyde (MDA), a product of lipid peroxidation, is used as an index of oxidative stress (Del Rio et al., 2005). MDA reacts with thiobarbituric acid (TBA) at high temperature and forms MDA-TBA adduct that can be measured colorimetrically using commercial assay kit (Cayman Chemical Company, USA) according to manufacturer's instructions and our study (El-Sayed et al., 2016).

2.13. Drugs and chemicals

Abn-cbd and O-1918 were purchased from Cayman Chemical (Cayman chemical company, Ann Arbor, MI); each drug was dissolved in methyl acetate solvent. To prepare an aqueous solution of each drug, methyl acetate solvent was volatilized and then each drug was dissolved in a mixture of (DMSO: PBS, 1:3; pH 7.2). Heparin (Elkins Sinn Inc., Cherry Hill, NJ) and sterile saline (B. Braun Medical Inc., Bethlehem, PA) were purchased from commercial vendors. STZ and DMSO were purchased from Sigma-Aldrich (St. Louis, MO).

2.14. Data analysis and statistics

Values of hemodynamic, ventricular functions as well as biochemical analyses were expressed as mean ± S.E.M. Statistical analyses were conducted using a one-way analysis of variance (ANOVA) with Tukey's post hoc test and Student's t test. Prism 5.0 software (GraphPad Software Inc., San Diego, CA) was used to perform statistical analysis and $P < 0.05$ was considered significant.

3. Results

3.1. Abn-cbd mitigated LV cardiac dysfunction and vagal dominance in diabetic rats

Conscious STZ-diabetic rats exhibited LV dysfunction observed as reductions ($P < 0.05$) in the LV contractility index, dP/dt_{max} (Fig. 1A) and LV relaxation index, dP/dt_{min} (Fig. 1B) along with elevation ($P < 0.05$) in LVEDP (Fig. 1C). Further, MAP (Fig. 2A) and HR (Fig. 2B) were reduced ($P < 0.05$), and frequency domain analysis showed increased ($P < 0.05$) HF_{RRI}, an index of vagal dominance (Fig. 3A), but no change in LF_{RRI}, index of sympathetic dominance (Fig. 3B), in diabetic rats. Abn-cbd partly alleviated the diabetes-induced reductions in dP/dt_{max} (Fig. 1A) and dP/dt_{min} (Fig. 1B) while markedly alleviated the increases in LVEDP (Fig. 1C) and HF_{RRI} (Fig. 3A) in diabetic rats. Further, abn-cbd treated diabetic rats still exhibited similar reductions in MAP (Fig. 2A) and HR (Fig. 2B) but showed reduction ($P < 0.05$) in sympathetic dominance, LF_{RRI} (Fig. 3B), compared with untreated STZ-diabetic rats. GPR18 blockade (O-1918) had no effect on the measured variables, but its concurrent administration with abn-cbd, abrogated the hemodynamic and sympathovagal effects of abn-cbd (Figs. 1–3).

3.2. Abn-cbd had no effect on impaired glycemic control or myocardial hypertrophy in diabetic rats

The STZ-diabetic rats exhibited elevation ($P < 0.05$) in fasting BGL, and reduction ($P < 0.05$) in plasma insulin levels (Table 1) along with higher ($P < 0.05$) HW/BW ratio, and lower ($P < 0.05$) BW, compared to non-diabetic rats (Table 2). Abn-cbd and/or O-1918 had no effect on these responses in diabetic rats (Tables 1 and 2).

3.3. Abn-cbd increased cardiac GPR18 expression and ADN levels and mitigated diabetes-induced reductions in circulating ADN and NOx levels

Abn-cbd partly (Fig. 4A) and fully (Fig. 5A) mitigated the reductions in circulating and cardiac ADN levels, respectively. Abn-cbd increased GPR18 expression (Fig. 5D) and prevented the reduction in circulating NOx (Fig. 4B), and the elevation in cardiac AdipoR1 expression (Fig. 5B), in diabetic rats. Cardiac AdipoR2 expression was not influenced by diabetes or the pharmacological interventions (Fig. 5C). Concurrent treatment with O-1918 abrogated the abn-cbd evoked effects on circulating and cardiac ADN (Figs. 4A and 5A), serum NOx (Fig. 4B) and cardiac GPR18 and AdipoR1 expressions (Figs. 5B and D).

3.4. Abn-cbd restored myocardial AKT, ERK1/2 and eNOS phosphorylation and mitigated the myocardial oxidative stress in diabetic rats

The diabetic rats' hearts exhibited reductions ($P < 0.05$) in Akt (Fig. 6A), ERK1/2 (Fig. 6B) and eNOS (Fig. 6C) phosphorylation, and in NO level (Fig. 7C) along with elevations ($P < 0.05$) in MDA level (Fig. 7A) and catalase activity (Fig. 7B). Abn-cbd alleviated these diabetes-induced biochemical responses (Figs. 6 and 7). Myocardial iNOS expression was not influenced by diabetes or by the pharmacological interventions (Fig. 6D). Concurrent O-1918 administration abrogated the abn-cbd evoked favorable effects on the phosphorylation of Akt, ERK1/2 and eNOS (Fig. 6), oxidative stress and NOx levels (Fig. 7)

in the hearts of diabetic rats. These molecular responses paralleled the favorable effects of abn-cbd on myocardial function and their abrogation by GPR18 blockade (Fig. 1).

4. Discussion

The present study demonstrates, for the first time, abn-cbd evoked alleviation of LV dysfunction and autonomic dysregulation in conscious diabetic rats. Further, we elucidated the molecular mechanisms of this action by demonstrating abn-cbd ability to: (i) partly and fully alleviate the reductions in circulating ADN and NO levels, respectively; (ii) increase myocardial GPR18 expression and ADN levels while reversing the elevation in cardiac AdipoR1 expression; (iii) restore myocardial Akt, ERK1/2 and eNOS phosphorylation, NO level, and redox status. Abn-cbd produced these salutary cardiac function and biochemical responses without correcting the diabetes-evoked impairment of glycemic control and LV hypertrophy. These effects are likely GPR18-mediated because concurrent administration of the same dose of the GPR18 antagonist (O-1918), with abn-cbd, abrogated these functional and biochemical responses. Collectively, these findings suggest that compromised GPR18 signaling contributes to myocardial oxidative stress and dysfunction, and yield clinically relevant insight into the molecular mechanisms implicated in the abn-cbd evoked rescue of the compromised LV function in a model of diabetes.

Our recent abn-cbd evoked hypotension and modest improvement of LV function in healthy rats (Matouk et al., 2017; Penumarti and Abdel-Rahman, 2014b) inferred a salutary cardiovascular role for GPR18 in models of human disease such as diabetes. Consistent with reported findings (Litwin et al., 1990), the STZ-diabetic model exhibits LV dysfunction (Fig. 1). Further, we reasoned that the diabetes associated reductions in circulating and myocardial ADN levels (Guo et al., 2007) could be offset by GPR18-mediated elevation in circulating and tissue ADN levels (Matouk et al., 2017; Penumarti and Abdel-Rahman, 2014b). In support of our scientific premise in STZ-diabetic rats, abn-cbd partly (Fig. 1A) and fully (Fig. 1C) alleviated LV contractility and LVDEP dysfunction, respectively; increased myocardial ADN and partly restored circulating ADN levels (Figs. 4A and 5A). Further, concurrent GPR18 blockade (O-1918) abolished these effects.

We first considered the possibility that abn-cbd alleviated the diabetes-evoked LV dysfunction (Fig. 1) by reversing the adverse cardiac effects of the STZ-induced impairment of glycemic control and/or cardiac hypertrophy observed here (Tables 1, 2) and in reported studies (Wang et al., 2013; Yu et al., 2008). Specifically, hyperglycemia (Brownlee, 2001) and hypoinsulinemia (Borges et al., 2006) contribute to myocardial dysfunction via oxidative stress and reduction in myocardial contractility, respectively. The finding that abn-cbd had no effect on the impaired glycemic control or the associated LV hypertrophy (Tables 1, 2) suggests glycemic control-independent mechanisms for the abn-cbd evoked cardioprotection in STZ-diabetic rats.

A viable mechanism for the abn-cbd evoked cardioprotection is likely GPR18-mediated and involves reversal of the increased vagal dominance (Fig. 3A) because the latter contributes to cardiac dysfunction, hypotension and bradycardia in STZ diabetic rats (Karakida et al., 1991) and in ethanol-treated rats (El-Mas and Abdel-Rahman, 2013). STZ-induced increase

in vagal dominance (Fig. 3A) is likely due to increased cardiac acetylcholine level and choline acetyltransferase activities (Akiyama et al., 1989). By contrast, others have reported increased sympathetic and decreased vagal activities in STZ diabetic rats (De Angelis et al., 2000; Howarth et al., 2005), which might explain the hypertension in reported studies (El-Sayed et al., 2016; Jackson and Carrier, 1981; Musial et al., 2013). It is also possible that tail cuff blood pressure measurements contributed to the modest hypertension in some of these reported studies. Notably, the modest hypotension observed, here (Fig. 2A) and in other studies (Jackson and Carrier, 1983) might also result from reduced cardiac output secondary to LV dysfunction (Fig. 1) (Ren and Bode, 2000) and from osmotic diuresis-evoked hypovolemia (Teshima et al., 2000).

It is noteworthy that despite the reversal of the diabetic-induced vagal dominance (Fig. 3A), the hypotension and bradycardia remained in abn-cbd treated diabetic rats (Fig. 2). This discrepancy might be explained by the reduction in sympathetic dominance (Fig. 3B), and by the increase in circulating NO levels, which likely resulted from abn-cbd evoked correction of the diabetes-induced reductions in circulating ADN levels (Fig. 4). This notion is supported by our findings in healthy rats (Matouk et al., 2017; Penumarti and Abdel-Rahman, 2014a) and by the NO-dependent vasorelaxation caused by cannabidiol in diabetic rats (Wheal et al., 2017). Collectively, these findings support an important role for the improved cardiac autonomic functions and increased NO and ADN in the abn-cbd evoked cardioprotection in diabetic rats.

To further understand the role of ADN and NO in the abn-cbd evoked mitigation of myocardial oxidative stress in diabetes (Fig. 7A); we investigated the responses of molecular modulators of oxidative stress in treated and untreated diabetic rats. These studies were built on the involvement of ADN-NOS signaling in the favorable cardiovascular effects caused by acute (Penumarti and Abdel-Rahman, 2014a) or chronic (Matouk et al., 2017) abn-cbd administration in healthy animals. Our scientific premise was further supported by the ADN-mediated reactive oxygen species reduction (Margaritis et al., 2013; Tao et al., 2007) and by enhancement of eNOS-derived NO level (Xi et al., 2005). Notably, diabetes is associated with reductions in circulating ADN levels (Fig. 4A) (Guo et al., 2007) and increased cardiac lipid peroxidation (Fig. 7A) (El-Sayed et al., 2016; Wang et al., 2013). The higher myocardial catalase activity in diabetic rats (Fig. 7B) might serve as a counterbalance for the increased myocardial oxidative stress (Bukan et al., 2004).

The abn-cbd evoked increase in ADN levels in diabetic rats (Figs. 4A and 5A), possibly resulting from GPR18 activation in adipocytes and cardiac ADN synthesis (Amisten et al., 2015), may improve cardiac function via activation of cardiac ADN receptors (Pineiro et al., 2005). The increased myocardial AdipoR1 expression (Fig. 5B) may be hypoinsulinemia (Table 1) mediated (Tsuchida et al., 2004) or a compensatory response to the reduced circulating ADN levels (Guo et al., 2007) in STZ diabetic rats (Fig. 4A). The latter possibility is confirmed by the reversal of the upregulated cardiac AdipoR1 (Fig. 5B) when circulating and cardiac ADN (Figs. 4A and 5A) levels were increased and partly restored, respectively, in abn-cbd treated diabetic rats. Cardiac AdipoR2 expression was not altered in STZ-diabetic rats (Fig. 5C) (Guo et al., 2007) or by abn-cbd (Fig. 5C). These molecular responses support an important role for AdipoR1 signaling in the abn-cbd evoked reduction

in myocardial reactive oxygen species (Figs. 7A and B) and cardioprotection in diabetic rats (Fig. 1) consistent with findings in other models of myocardial dysfunction (Tao et al., 2007). Further, abn-cbd evoked increase in myocardial GPR18 level (Fig. 5D) paralleled the favorable cardiovascular and autonomic effects (Figs. 1 and 3) in diabetic rats.

Reported findings including ours linked abn-cbd evoked favorable effects to the activation of the PI3K/Akt/ERK1/2 cascade via GPR18 mediated increase in ADN levels (Matouk et al., 2017; Offertaler et al., 2003; Penumarti and Abdel-Rahman, 2014a; Xi et al., 2005). Therefore, ADN contribution to the restoration of Akt and ERK1/2 phosphorylation (Figs. 6 A and B) may contribute to the abn-cbd evoked cardioprotection in diabetic rats (Fig. 1) via the following mechanisms: (i) alleviation of oxidative stress (Figs. 7A and B) (Penumarti and Abdel-Rahman, 2014a), and inhibition of apoptosis (Fujio et al., 2000), (ii) stimulation of insulin mediated glucose uptake (Kohn et al., 1996), and (iii) activation of eNOS (Matouk et al., 2017; Penumarti and Abdel-Rahman, 2014a; Xi et al., 2005). Collectively, these findings highlight restorations of myocardial Akt, ERK1/2 and eNOS phosphorylation (Fig. 6) as important mechanisms for cardiac NO restoration in abn-cbd treated diabetic rats (Fig. 7C) because cardiac iNOS expression was not influenced by diabetes or by the pharmacological interventions (Fig. 6D).

It is important to comment on two important issues when interpreting the present findings. First, the contradictory reports on ADN contribution to cardiac function. The association between reduced ADN levels (Figs. 4A and 5A) and myocardial and autonomic dysfunction in diabetes (Figs. 1 and 3) (Guo et al., 2012) along with the recovery of cardiac ADN levels and cardiac function in abn-cbd treated diabetic rats are consistent with a favorable cardiac effect for ADN via its anti-inflammatory function (Antoniades et al., 2009). By contrast, increased ADN levels are associated with adverse cardiovascular events (Witberg et al., 2016). Therefore, future studies using ADN receptor knockout mice are warranted to ascertain ADN role in diabetic rats. Second, we acknowledge that abn-cbd can activate other receptors such as GPR55 (Johns et al., 2007; McHugh et al., 2010; Ryberg et al., 2007) while proposing GPR18 involvement in the abn-cbd evoked hemodynamic and biochemical responses. Our premise is supported by the findings that GPR55 does not mediate abn-cbd evoked hypotension (Caldwell et al., 2013; Johns et al., 2007), and the selective GPR55 (O-1602) does not lower BP (Walsh et al., 2015). Future studies are needed to address this debated issue.

5. Conclusion

The present study is the first to demonstrate the abn-cbd-mediated favorable cardiovascular effects in diabetes. Abn-cbd increased myocardial GPR18 expression and mitigated the LV dysfunction, at least partly, via reversing the diabetes-induced: (i) increases in cardiac vagal dominance and myocardial oxidative stress, and (ii) reductions in circulating NO, ADN levels, and in the phosphorylation of myocardial eNOS, Akt and ERK1/2. Our conclusions gain credence from the pharmacological evidence that concurrent GPR18 blockade (O-1918) abrogated the salutary biochemical and cardiovascular effects of abn-cbd in diabetic rats. Finally, the present findings in a diabetic rat model with established cardiovascular

anomalies have clinical ramifications by proposing new adjunctive therapeutics to improve cardiovascular function in diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Akiyama N, Okumura K, Watanabe Y, Hashimoto H, Ito T, Ogawa K, Satake T. Altered acetylcholine and norepinephrine concentrations in diabetic rat hearts. Role of parasympathetic nervous system in diabetic cardiomyopathy. Diabetes. 1989; 38:231–236. [PubMed: 2644143]

Amisten S, Neville M, Hawkes R, Persaud SJ, Karpe F, Salehi A. An atlas of G-protein coupled receptor expression and function in human subcutaneous adipose tissue. Pharmacol Ther. 2015; 146:61–93. [PubMed: 25242198]

Antoniades C, Antonopoulos AS, Tousoulis D, Stefanadis C. Adiponectin: from obesity to cardiovascular disease. Obes Rev. 2009; 10:269–279. [PubMed: 19389061]

Appel ML, Berger RD, Saul JP, Smith JM, Cohen RJ. Beat to beat variability in cardiovascular variables: noise or music? J Am Coll Cardiol. 1989; 14:1139–1148. [PubMed: 2681319]

Borges GR, de Oliveira M, Salgado HC, Fazan R Jr. Myocardial performance in conscious streptozotocin diabetic rats. Cardiovasc Diabetol. 2006; 5:26. [PubMed: 17144912]

Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001; 414:813–820. [PubMed: 11742414]

Bukan N, Sancak B, Bilgihan A, Kosova F, Bugdayci G, Altan N. The effects of the sulfonylurea glyburide on glutathione peroxidase, superoxide dismutase and catalase activities in the heart tissue of streptozotocin-induced diabetic rat. Methods Find Exp Clin Pharmacol. 2004; 26:519–522. [PubMed: 15538541]

Burstein SH, McQuain CA, Ross AH, Salmonsen RA, Zurier RE. Resolution of inflammation by N-arachidonoylglycine. J Cell Biochem. 2011; 112:3227–3233. [PubMed: 21732409]

Caldwell MD, Hu SS, Viswanathan S, Bradshaw H, Kelly ME, Straiker A. A GPR18-based signalling system regulates IOP in murine eye. Br J Pharmacol. 2013; 169:834–843. [PubMed: 23461720]

De Angelis KL, Oliveira AR, Dall'Ago P, Peixoto LR, Gadonski G, Lacchini S, Fernandes TG, Irigoyen MC. Effects of exercise training on autonomic and myocardial dysfunction in streptozotocin-diabetic rats. Braz J Med Biol Res. 2000; 33:635–641. [PubMed: 10829091]

Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis. 2005; 15:316–328. [PubMed: 16054557]

El-Mas MM, Abdel-Rahman AA. Cardiovascular autonomic modulation by nitric oxide synthases accounts for the augmented enalapril-evoked hypotension in ethanol-fed female rats. Alcohol. 2013; 47:339–346. [PubMed: 23683529]

El-Sayed SS, Zakaria MN, Abdel-Ghany RH, Abdel-Rahman AA. Cystathionine-gamma lyase-derived hydrogen sulfide mediates the cardiovascular protective effects of moxonidine in diabetic rats. Eur J Pharmacol. 2016; 783:73–84. [PubMed: 27138707]

Frankenfeld SP, de Oliveira LP, Ignacio DL, Coelho RG, Mattos MN, Ferreira AC, Carvalho DP, Fortunato RS. Nandrolone decanoate inhibits gluconeogenesis and decreases fasting glucose in Wistar male rats. J Endocrinol. 2014; 220:143–153. [PubMed: 24403377]

Fujio Y, Nguyen T, Wencker D, Kitsis RN, Walsh K. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. Circulation. 2000; 101:660–667. [PubMed: 10673259]

Gantz I, Muraoka A, Yang YK, Samuelson LC, Zimmerman EM, Cook H, Yamada T. Cloning and chromosomal localization of a gene (GPR18) encoding a novel seven transmembrane receptor highly expressed in spleen and testis. Genomics. 1997; 42:462–466. [PubMed: 9205118]

Guo Z, Xia Z, Yuen VG, McNeill JH. Cardiac expression of adiponectin and its receptors in streptozotocin-induced diabetic rats. Metabolism. 2007; 56:1363–1371. [PubMed: 17884446]

Guo Z, Zhang R, Li J, Xu G. Effect of telmisartan on the expression of adiponectin receptors and nicotinamide adenine dinucleotide phosphate oxidase in the heart and aorta in type 2 diabetic rats. Cardiovasc Diabetol. 2012; 11:94. [PubMed: 22873349]

Horvath B, Mukhopadhyay P, Hasko G, Pacher P. The endocannabinoid system and plant-derived cannabinoids in diabetes and diabetic complications. Am J Pathol. 2012; 180:432–442. [PubMed: 22155112]

Howarth FC, Jacobson M, Shafiullah M, Adeghate E. Long-term effects of streptozotocin-induced diabetes on the electrocardiogram, physical activity and body temperature in rats. Exp Physiol. 2005; 90:827–835. [PubMed: 16091403]

Ibrahim BM, Abdel-Rahman AA. A pivotal role for enhanced brainstem Orexin receptor 1 signaling in the central cannabinoid receptor 1-mediated pressor response in conscious rats. Brain Res. 2015; 1622:51–63. [PubMed: 26096126]

Ibrahim BM, Fan M, Abdel-Rahman AA. Oxidative stress and autonomic dysregulation contribute to the acute time-dependent myocardial depressant effect of ethanol in conscious female rats. Alcohol Clin Exp Res. 2014; 38:1205–1215. [PubMed: 24754626]

Jackson CV, Carrier GO. Supersensitivity of isolated mesenteric arteries to noradrenaline in the long-term experimental diabetic rat. J Auton Pharmacol. 1981; 1:399–405. [PubMed: 6123513]

Jackson CV, Carrier GO. Influence of short-term experimental diabetes on blood pressure and heart rate in response to norepinephrine and angiotensin II in the conscious rat. J Cardiovasc Pharmacol. 1983; 5:260–265. [PubMed: 6188900]

Johns DG, Behm DJ, Walker DJ, Ao Z, Shapland EM, Daniels DA, Riddick M, Dowell S, Staton PC, Green P, Shabon U, Bao W, Aiyar N, Yue TL, Brown AJ, Morrison AD, Douglas SA. The novel endocannabinoid receptor GPR55 is activated by atypical cannabinoids but does not mediate their vasodilator effects. Br J Pharmacol. 2007; 152:825–831. [PubMed: 17704827]

Karakida T, Yamazaki Y, Homma S. Blood pressure and heart rate changes in streptozotocin diabetic rats, with special reference to postural hypotension. Jpn J Physiol. 1991; 41:589–603. [PubMed: 1791661]

Kohn AD, Summers SA, Birnbaum MJ, Roth RA. Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. J Biol Chem. 1996; 271:31372–31378. [PubMed: 8940145]

Kohno M, Hasegawa H, Inoue A, Muraoka M, Miyazaki T, Oka K, Yasukawa M. Identification of N-arachidonylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. Biochem Biophys Res Commun. 2006; 347:827–832. [PubMed: 16844083]

Litwin SE, Raya TE, Anderson PG, Daugherty S, Goldman S. Abnormal cardiac function in the streptozotocin-diabetic rat. Changes in active and passive properties of the left ventricle. J Clin Invest. 1990; 86:481–488. [PubMed: 2200804]

Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. Circulation. 1991; 84:482–492. [PubMed: 1860193]

Margaritis M, Antonopoulos AS, Digby J, Lee R, Reilly S, Coutinho P, Shirodaria C, Sayeed R, Petrou M, De Silva R, Jalilzadeh S, Demosthenous M, Bakogiannis C, Tousoulis D, Stefanadis C, Choudhury RP, Casadei B, Channon KM, Antoniades C. Interactions between vascular wall and perivascular adipose tissue reveal novel roles for adiponectin in the regulation of endothelial nitric oxide synthase function in human vessels. Circulation. 2013; 127:2209–2221. [PubMed: 23625959]

Matouk AI, Taye A, El-Moselhy MA, Heeba GH, Abdel-Rahman AA. The Effect of Chronic Activation of the Novel Endocannabinoid Receptor GPR18 on Myocardial Function and Blood Pressure in Conscious Rats. J Cardiovasc Pharmacol. 2017; 69:23–33. [PubMed: 27676325]

McHugh D, Hu SS, Rimmerman N, Juknat A, Vogel Z, Walker JM, Bradshaw HB. N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. BMC Neurosci. 2010; 11:44. [PubMed: 20346144]

Musial DC, da Silva ED Junior, da Silva RM, Miranda-Ferreira R, Lima-Landman MT, Jurkiewicz A, Garcia AG, Jurkiewicz NH. Increase of angiotensin-converting enzyme activity and peripheral sympathetic dysfunction could contribute to hypertension development in streptozotocin-induced diabetic rats. Diab Vasc Dis Res. 2013; 10:498–504. [PubMed: 23975725]

Nakamaki S, Satoh H, Kudoh A, Hayashi Y, Hirai H, Watanabe T. Adiponectin reduces proteinuria in streptozotocin-induced diabetic Wistar rats. Exp Biol Med (Maywood). 2011; 236:614–620. [PubMed: 21521713]

Offertaler L, Mo FM, Batkai S, Liu J, Begg M, Razdan RK, Martin BR, Bukoski RD, Kunos G. Selective ligands and cellular effectors of a G protein-coupled endothelial cannabinoid receptor. Mol Pharmacol. 2003; 63:699–705. [PubMed: 12606780]

Pae EK, Kim G. Insulin production hampered by intermittent hypoxia via impaired zinc homeostasis. PLoS One. 2014; 9:e90192. [PubMed: 24587273]

Parmar N, Ho WS. N-arachidonoyl glycine, an endogenous lipid that acts as a vasorelaxant via nitric oxide and large conductance calcium-activated potassium channels. Br J Pharmacol. 2010; 160:594–603. [PubMed: 20136843]

Penumarti A, Abdel-Rahman AA. Neuronal nitric oxide synthase-dependent elevation in adiponectin in the rostral ventrolateral medulla underlies g protein-coupled receptor 18-mediated hypotension in conscious rats. J Pharmacol Exp Ther. 2014a; 351:44–53. [PubMed: 25100751]

Penumarti A, Abdel-Rahman AA. The novel endocannabinoid receptor GPR18 is expressed in the rostral ventrolateral medulla and exerts tonic restraining influence on blood pressure. J Pharmacol Exp Ther. 2014b; 349:29–38. [PubMed: 24431468]

Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB(1) and CB(2). Pharmacol Rev. 2010; 62:588–631. [PubMed: 21079038]

Pineiro R, Iglesias MJ, Gallego R, Raghay K, Eiras S, Rubio J, Dieguez C, Gualillo O, Gonzalez-Juanatey JR, Lago F. Adiponectin is synthesized and secreted by human and murine cardiomyocytes. FEBS Lett. 2005; 579:5163–5169. [PubMed: 16140297]

Rajesh M, Batkai S, Kechrid M, Mukhopadhyay P, Lee WS, Horvath B, Holovac E, Cinar R, Liaudet L, Mackie K, Hasko G, Pacher P. Cannabinoid 1 receptor promotes cardiac dysfunction, oxidative stress, inflammation, and fibrosis in diabetic cardiomyopathy. Diabetes. 2012; 61:716–727. [PubMed: 22315315]

Ren J, Bode AM. Altered cardiac excitation-contraction coupling in ventricular myocytes from spontaneously diabetic BB rats. Am J Physiol Heart Circ Physiol. 2000; 279:H238–244. [PubMed: 10899062]

Ross RA. The enigmatic pharmacology of GPR55. Trends Pharmacol Sci. 2009; 30:156–163. [PubMed: 19233486]

Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, Elebring T, Nilsson K, Drmota T, Greasley PJ. The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol. 2007; 152:1092–1101. [PubMed: 17876302]

Schnedl WJ, Ferber S, Johnson JH, Newgard CB. STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. Diabetes. 1994; 43:1326–1333. [PubMed: 7926307]

Shaltout HA, Abdel-Rahman AA. Mechanism of fatty acids induced suppression of cardiovascular reflexes in rats. J Pharmacol Exp Ther. 2005; 314:1328–1337. [PubMed: 15937146]

Tao L, Gao E, Jiao X, Yuan Y, Li S, Christopher TA, Lopez BL, Koch W, Chan L, Goldstein BJ, Ma XL. Adiponectin cardioprotection after myocardial ischemia/reperfusion involves the reduction of oxidative/nitrative stress. Circulation. 2007; 115:1408–1416. [PubMed: 17339545]

Teshima Y, Takahashi N, Saikawa T, Hara M, Yasunaga S, Hidaka S, Sakata T. Diminished expression of sarcoplasmic reticulum Ca(2+)-ATPase and ryanodine sensitive Ca(2+)Channel mRNA in streptozotocin-induced diabetic rat heart. J Mol Cell Cardiol. 2000; 32:655–664. [PubMed: 10756121]

Tsuchida A, Yamauchi T, Ito Y, Hada Y, Maki T, Takekawa S, Kamon J, Kobayashi M, Suzuki R, Hara K, Kubota N, Terauchi Y, Froguel P, Nakae J, Kasuga M, Accili D, Tobe K, Ueki K, Nagai R, Kadowaki T. Insulin/Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. J Biol Chem. 2004; 279:30817–30822. [PubMed: 15123605]

Van Linthout S, Spillmann F, Riad A, Trimpert C, Lievens J, Meloni M, Escher F, Filenberg E, Demir O, Li J, Shakibaei M, Schimke I, Staudt A, Felix SB, Schultheiss HP, De Geest B, Tschope C. Human apolipoprotein A-I gene transfer reduces the development of experimental diabetic cardiomyopathy. Circulation. 2008; 117:1563–1573. [PubMed: 18332268]

Varga K, Lake K, Martin BR, Kunos G. Novel antagonist implicates the CB1 cannabinoid receptor in the hypotensive action of anandamide. Eur J Pharmacol. 1995; 278:279–283. [PubMed: 7589169]

Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. Circulation. 2007; 115:387–397. [PubMed: 17242296]

Walsh SK, Hepburn CY, Keown O, Astrand A, Lindblom A, Ryberg E, Hjorth S, Leslie SJ, Greasley PJ, Wainwright CL. Pharmacological profiling of the hemodynamic effects of cannabinoid ligands: a combined in vitro and in vivo approach. Pharmacol Res Perspect. 2015; 3:e00143. [PubMed: 26236485]

Wang GG, Li W, Lu XH, Zhao X, Xu L. Taurine attenuates oxidative stress and alleviates cardiac failure in type I diabetic rats. Croat Med J. 2013; 54:171–179. [PubMed: 23630144]

Wheal AJ, Jadoon K, Randall MD, O'Sullivan SE. In Vivo Cannabidiol Treatment Improves Endothelium-Dependent Vasorelaxation in Mesenteric Arteries of Zucker Diabetic Fatty Rats. Front Pharmacol. 2017; 8:248. [PubMed: 28572770]

Witberg G, Ayers CR, Turer AT, Lev E, Kornowski R, de Lemos J, Neeland IJ. Relation of Adiponectin to All-Cause Mortality, Cardiovascular Mortality, and Major Adverse Cardiovascular Events (from the Dallas Heart Study). Am J Cardiol. 2016; 117:574–579. [PubMed: 26800774]

Xi W, Satoh H, Kase H, Suzuki K, Hattori Y. Stimulated HSP90 binding to eNOS and activation of the PI3-Akt pathway contribute to globular adiponectin-induced NO production: vasorelaxation in response to globular adiponectin. Biochem Biophys Res Commun. 2005; 332:200–205. [PubMed: 15896318]

Yu BC, Chang CK, Ou HY, Cheng KC, Cheng JT. Decrease of peroxisome proliferator-activated receptor delta expression in cardiomyopathy of streptozotocin-induced diabetic rats. Cardiovasc Res. 2008; 80:78–87. [PubMed: 18573863]

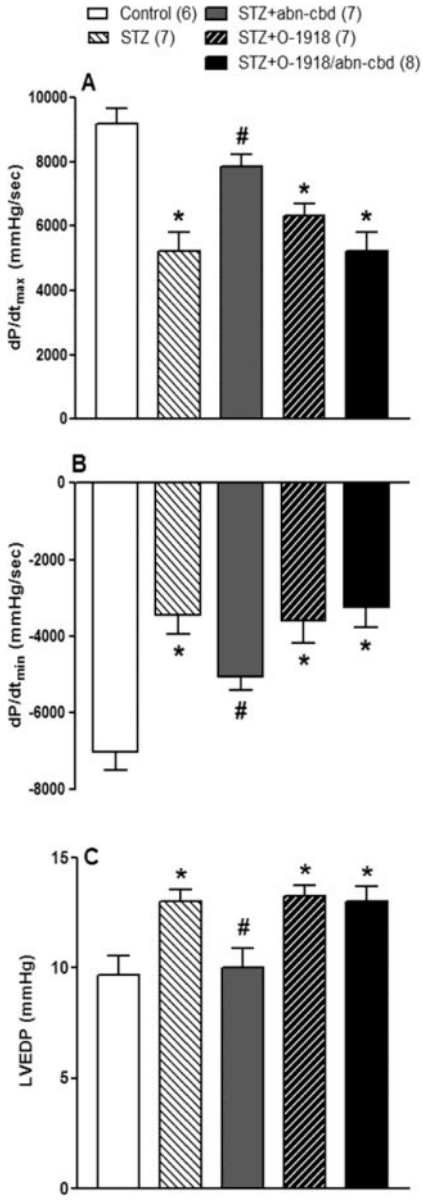

Figure 1.

Effects of 2-week administration of abn-cbd, O-1918 (100 μg/kg/day, i.p, each) or their combination, starting 4 weeks after diabetes induction, on left ventricular (LV) maximum contraction velocity (dP/dt_{max}; A), LV maximum relaxation velocity (dP/dt_{min}; B), and LV end diastolic pressure (LVEDP; C) in conscious male diabetic rats. Numbers of rats per group is shown in parentheses. Values are mean ± S.E.M. *P < 0.05 versus non-diabetic control rats and #P < 0.05 versus vehicle-treated diabetic control rats.

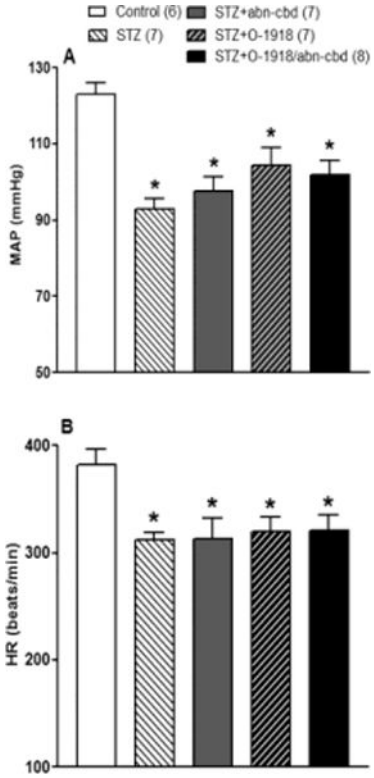

Figure 2.

Effects of 2-week administration of abn-cbd, O-1918 (100 μg/kg/day, i.p, each) or their combination, starting 4 weeks after diabetes induction, on mean arterial pressure (MAP; A), and heart rate (HR; B) in conscious male diabetic rats. Numbers of rats per group is shown in parentheses. Values are mean ± S.E.M. *P < 0.05 versus non-diabetic control rats.

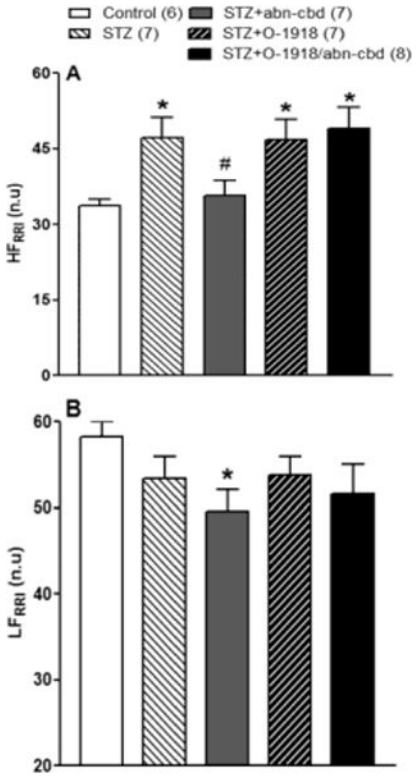

Figure 3.

High-frequency (0.75–3 Hz) component (A) and low-frequency (0.25–0.75 Hz) component (B) of spectral analysis of the R-R interval (RRI), index of cardiac vagal and sympathetic dominance, respectively, in conscious male diabetic rats treated for 2 weeks with abn-cbd, O-1918 (100 μg/kg/day, i.p, each) or their combination starting 4 weeks after diabetes induction. Numbers of rats per group is shown in parentheses. Values are mean ± S.E.M. *P < 0.05 versus non-diabetic control rats and #P < 0.05 versus vehicle-treated diabetic control rats.

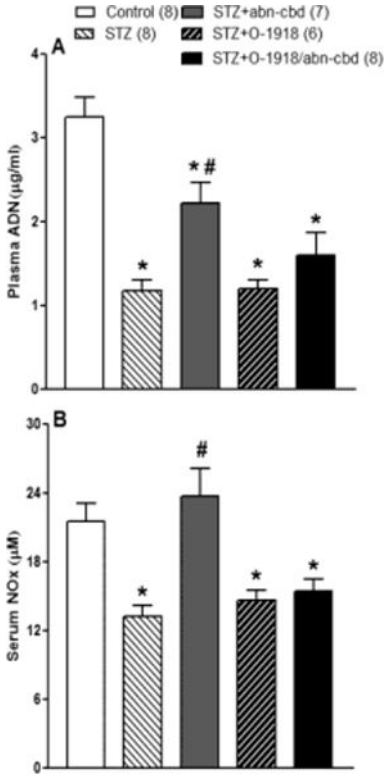

Figure 4.

Effects of 2-week administration of abn-cbd, O-1918 (100 µg/kg/day, i.p, each) or their combination, starting 4 weeks after diabetes induction, on the levels of plasma adiponectin (A) and serum nitrate/nitrite (NOx; B) in male diabetic rats. Numbers of rats per group is shown in parentheses. Values are mean ± S.E.M. *P < 0.05 versus non-diabetic control rats, and #P < 0.05 versus vehicle-treated diabetic control rats.

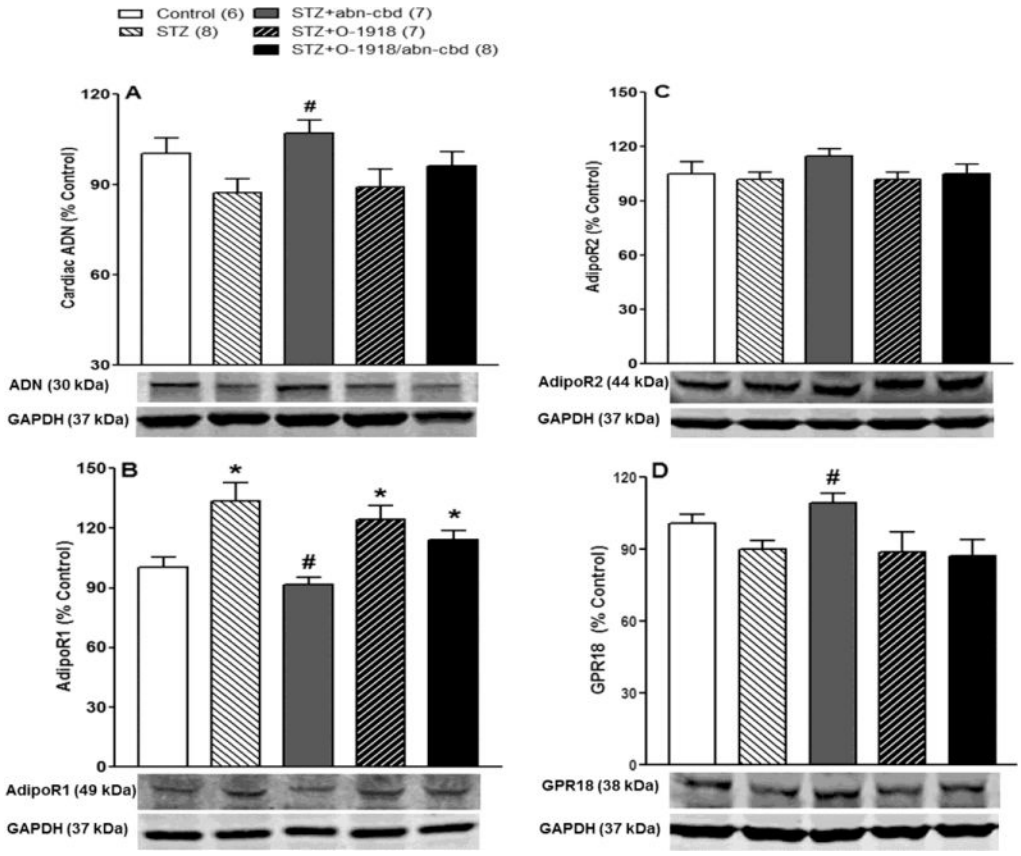

Figure 5.

Western blot analyses showing the effect of 2-week administration of abn-cbd, O-1918 (100 μg/kg/day, i.p, each) or their combination, starting 4 weeks after diabetes induction, on myocardial adiponectin (ADN; A), adiponectin receptor-1 (AdipoR1; B), adiponectin receptor-2 (AdipoR2; C) and GPR18 expression (D) in male diabetic rats. ADN, AdipoR1, AdipoR2 and GPR18 levels were normalized to GAPDH. Protein expression was presented as percent of control. Numbers of rats per group is shown in parentheses. Values are mean ± S.E.M. *P < 0.05 versus non-diabetic control rats and #P < 0.05 versus vehicle-treated diabetic control rats.

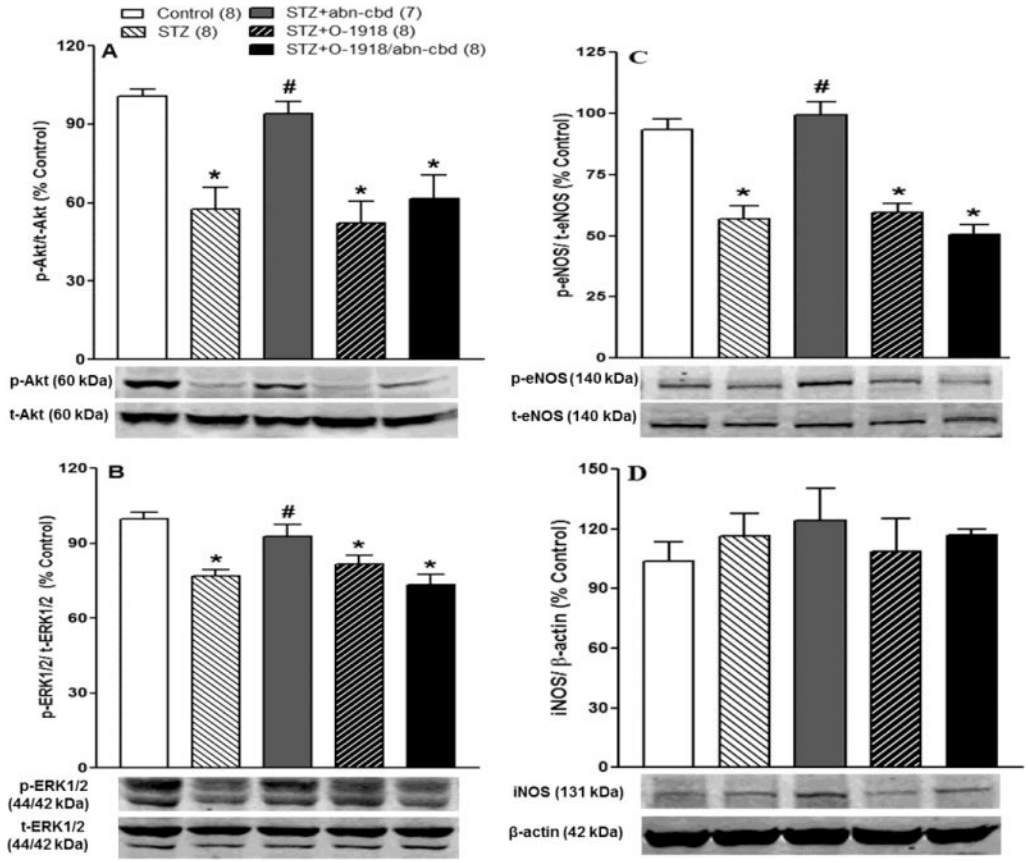

Figure 6.

Western blot analyses showing the effect of 2-week administration of abn-cbd, O-1918 (100 μg/kg/day, i.p, each) or their combination, starting 4 weeks after diabetes induction on myocardial: p-Akt (A), p-ERK1/2 (B), p-eNOS (C) and inducible NOS (iNOS) expression (D) in male diabetic rats. Data represent the ratio of p-Akt, p-ERK1/2 or p-eNOS to the corresponding total protein, whereas the iNOS levels are normalized to β-actin. Protein expression was presented as percent of control. Numbers of rats per group is shown in parentheses. Values are mean ± S.E.M. *P < 0.05 versus non-diabetic rats and #P < 0.05 versus vehicle-treated diabetic control rats.

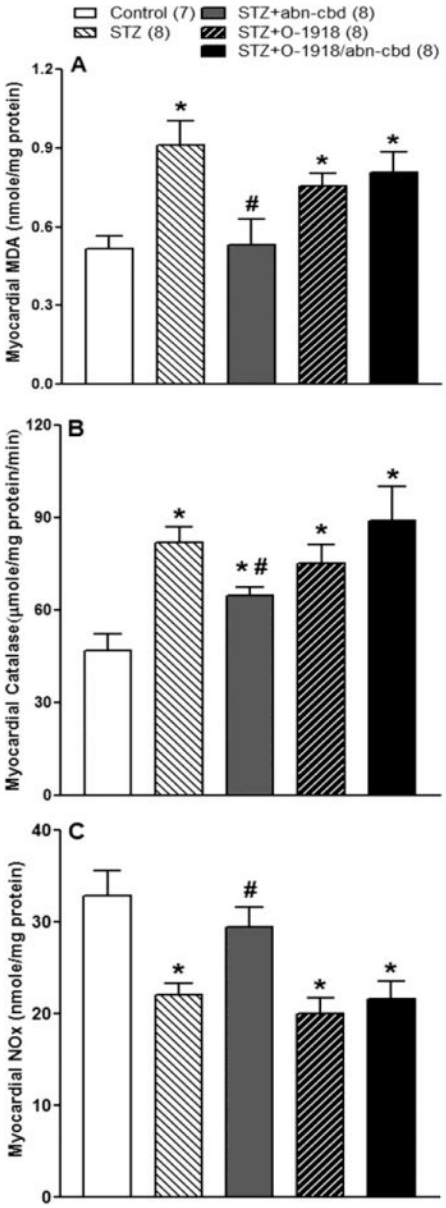

Figure 7.

Effects of 2-week administration of abn-cbd, O-1918 (100 µg/kg/day, i.p, each) or their combination, starting 4 weeks after diabetes induction, on the myocardial levels of malondialdehyde (MDA; A), the antioxidant enzyme catalase (B) and nitrate/nitrite (NOx; C) in male diabetic rats. Numbers of rats per group is shown in parentheses. Values are mean ± S.E.M. *P < 0.05 versus non-diabetic control rats, and #P < 0.05 versus vehicle-treated diabetic control rats.

Table 1

Effects of 2-week administration of abn-cbd, O-1918 (100 μg/kg/day, i.p, each) or their combination, starting 4 weeks after diabetes induction, on fasting blood glucose level (BGL), oral glucose tolerance test (OGTT) total area under the curve (AUC) per 120 min and plasma insulin levels in male diabetic rats.

N	Fasting BGL (mg/dl)	AUC	Plasma insulin (ng/ml)
Non-diabetic control (8)	73.4 ± 3.311	11490 ± 109.5	1.73 ± 0.220
STZ diabetic vehicle (6)	437.2 ± 31.34[a]	53150 ± 2269[a]	0.46 ± 0.057[a]
STZ diabetic + abn-cbd (7)	423.9 ± 30.24[a]	47000 ± 3235[a]	0.48 ± 0.072[a]
STZ diabetic + O-1918 (7)	414.3 ± 25.01[a]	51960 ± 2210[a]	0.49 ± 0.179[a]
STZ diabetic + O-1918/abn-cbd (7)	445.9 ± 29.05[a]	51660 ± 2321[a]	0.39 ± 0.046[a]

Numbers of rats per group is shown in parentheses. Values are mean ± S.E.M.

[a] $P < 0.05$ versus non-diabetic control rats.

Table 2

Effects of 2-week administration of abn-cbd, O-1918 (100 μg/kg/day, i.p, each) or their combination, starting 4 weeks after diabetes induction, on heart weight (HW), body weight (BW) and HW/BW ratio “an index of cardiac hypertrophy” in male diabetic rats.

N	Heart weight (g)	Body weight (g)	HW/BW(g/kg)
Non-diabetic control (8)	1.22 ± 0.058	438.8 ± 13.3	2.83 ± 0.07
STZ diabetic (vehicle) (7)	1.12 ± 0.029	318.4 ± 11.2[a]	3.52 ± 0.11[a]
STZ diabetic + abn-cbd (7)	1.17 ± 0.054	323 ± 14.9[a]	3.62 ± 0.093[a]
STZ diabetic + O-1918 (7)	1.18 ± 0.075	336.9 ± 14.2[a]	3.62 ± 0.14[a]
STZ diabetic + O-1918/abn-cbd (8)	1.13 ± 0.062	323.8 ± 9.6[a]	3.27 ± 0.18[a]

Numbers of rats per group is shown in parentheses. Values are mean ± S.E.M.

[a] $P < 0.05$ versus non-diabetic control rats.