Cannabinoid 1 Receptor Promotes Cardiac Dysfunction, Oxidative Stress, Inflammation, and Fibrosis in Diabetic Cardiomyopathy

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Endocannabinoids and cannabinoid 1 (CB1) receptors have been implicated in cardiac dysfunction, inflammation, and cell death associated with various forms of shock, heart failure, and atherosclerosis, in addition to their recognized role in the development of various cardiovascular risk factors in obesity/metabolic syndrome and diabetes. In this study, we explored the role of CB1 receptors in myocardial dysfunction, inflammation, oxidative/nitrative stress, cell death, and interrelated signaling pathways, using a mouse model of type 1 diabetic cardiomyopathy. Diabetic cardiomyopathy was characterized by increased myocardial endocannabinoid anandamide levels, oxidative/nitrative stress, activation of p38Jun NH2-terminal kinase (JNK) mitogen-activated protein kinases (MAPKs), enhanced inflammation (tumor necrosis factor-α, interleukin-1β, cyclooxygenase 2, intracellular adhesion molecule 1, and vascular cell adhesion molecule 1), increased expression of CB1, advanced glycation end product (AGE) and angiotensin II type 1 receptors (receptor for advanced glycation end product [RAGE], angiotensin II receptor type 1 [AT1R]), p47(phox) NADPH oxidase subunit, β-myosin heavy chain isozyme switch, accumulation of AGE, fibrosis, and decreased expression of sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA2a). Pharmacological inhibition or genetic deletion of CB1 receptors attenuated the diabetes-induced cardiac dysfunction and the above-mentioned pathological alterations. Activation of CB1 receptors by endocannabinoids may play an important role in the pathogenesis of diabetic cardiomyopathy by facilitating MAPK activation, AT1R expression/signaling, AGE accumulation, oxidative/nitrative stress, inflammation, and fibrosis. Conversely, CB1 receptor inhibition may be beneficial in the treatment of diabetic cardiovascular complications.

In diabetic patients, cardiovascular complications represent the principal cause of morbidity and mortality. Myocardial left ventricular (LV) dysfunction (both diastolic and later systolic) independent of atherosclerosis and coronary artery disease has been well documented in both humans and animals (1,2). The mechanisms of diabetic cardiomyopathy are multifaceted, involving increased oxidative/nitrosative stress (3–6), accumulation of advanced glycation end products (AGEs) (7–9), enhanced receptor for advanced glycation end product (RAGE) and angiotensin II receptor type 1 (AT1R) signaling (3,7–9), activation of various proinflammatory and cell death signaling pathways [e.g., poly(ADP-ribose) polymerase (PARP)], mitogen-activated protein kinases (MAPKs) (10,14–16), coupled with consequent changes in the composition of extracellular matrix with enhanced cardiac fibrosis (13,16), myosin heavy chain (MHC) isoform switch (17), and decreased activity of sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA2a) (18–20), just to mention a few.

Recent preclinical and clinical studies have importantly implicated endocannabinoids (novel lipid mediators) and cannabinoid 1 (CB1) receptors in the regulation of food intake, energy balance, and metabolism (21–23). CB1 receptor (CB1R) inhibition with rimonabant (SR141716/SR1) demonstrated multiple beneficial effects on metabolic and inflammatory markers both in obese and/or type 2 diabetic patients, as well as in various preclinical disease models (21,23). CB1 receptors are predominantly expressed in the central nervous system (21), but are also present in cardiovascular and virtually all other peripheral tissues, albeit at much lower levels (24,25). In the cardiovascular system, CB1 activation by endocannabinoids or synthetic ligands leads to complex cardiovascular depressive effects, implicated in the cardiovascular collapse associated with various forms of shock (21) and heart failure (26–28). CB1R activation in coronary artery endothelial cells (29), cardiomyocytes (26,27), and inflammatory cells (28,30) mediates MAPK activation, reactive oxygen species (ROS) generation, and inflammatory response promoting atherosclerosis (31) and cardiac dysfunction (27,28). Furthermore, elevated endocannabinoid plasma levels have recently been associated with coronary circulatory dysfunction in human obesity (32), and CB1R blockade or its genetic deletion attenuated proteinuria and/or vascular inflammation and cell death in experimental models of type 1 diabetic nephropathy (33) and/or retinopathy (34). Beneficial effect of CB1 blockade has also been reported in rodent models of type 1 diabetic neuropathy and in various high glucose–induced in vitro experimental paradigms (rev. in 35).

In this study, we investigated the potential role of the endocannabinoids and CB1R in the pathogenesis of type 1 diabetic cardiomyopathy using selective CB1R inhibitors or CB1 knockout mice. Our results demonstrate that pharmacological inhibition or genetic deletion of CB1 attenuates...
cardiac dysfunction, oxidative stress, inflammation, and fibrosis in diabetic mice.

**RESEARCH DESIGN AND METHODS**

**Animals and treatment.** Animal protocols used in this study adhered to the National Institutes of Health (NIH) guidelines and were approved by the Institutional Animal Care and Use Committee of the National Institute on Alcohol Abuse and Alcoholism (NIAAA). Diabetes was induced in 8- to 12-week-old C57/Bl6J (WT) mice (male; The Jackson Laboratories, Bar Harbor, ME) or CB1R−/−, CB1R+/+ mice (on C57/Bl6J background; Intramural Research Program of NIH/NIAAA, Bethesda, MD) by an intraperitoneal administration of streptozotocin (STZ) (Sigma, St. Louis, MO) at the dose of 50 mg/kg dissolved in 100 mM/L citrate buffer, pH 4.5, for 5 consecutive days as described (16). After 5 days, the blood glucose levels were measured using an Ascensia Counter Glucometer (Bayer HealthCare, Tarrytown, NY) by mandibular puncture blood sampling. Only mice that had blood glucose values >250 mg/dL were used for the study. Control animals were administered the same volume of citrate buffer, and all mice had access to food and water ad libitum. Diabetes was allowed to develop further for 1 additional week before animals were treated for 11 weeks with the selective CB1R antagonists SR141716A/rimonabant and AM281; 10 mg/kg i.p. daily; National Institute on Drug Abuse Drug Supply (26,36). A/D converter (AD Instruments, Mountain View, CA), as previously described in mice anesthetized with 2% isoflurane (Supplementary Fig. 6).

**RESULTS**

**Metabolic variables.** Induction of diabetes by multiple doses of STZ led to reduction in the body weights with increase in the blood glucose levels in WT and CB1R−/− CB1R+/+ mice, respectively (Supplementary Figs. 1, 2A, and 2B). However, the blood glucose levels were not different during the course of the 12-week study period in CB1R−/− and CB1R+/+ mice or in WT mice treated with vehicle or CB1R antagonists SR141716 (SR1) and AM281 (AM) (Supplementary Figs. 1A and 2A). Similarly, there were no significant differences in the HbA1c levels (Supplementary Figs. 1C and 2C) and the pancreatic insulin content among corresponding groups (Supplementary Figs. 1D and 2D), respectively.

**Diabetes increases myocardial CB1R expression and endocannabinoid anandamide levels:** Improved diabetes-induced cardiac dysfunction in CB1R−/− mice. Diabetic cardiomyopathy was associated with enhanced LV CB1R expression and anandamide (also known as N-arachidonylethanolamide; AEA) levels compared with WT mice (Fig. 1A and D). However, the blood glucose levels were not different during the course of the 12-week study period in CB1R−/− and CB1R+/+ mice or in WT mice treated with vehicle or CB1R antagonists SR141716 (SR1) and AM281 (AM) (Supplementary Figs. 1A and 2A). Similarly, there were no significant differences in the HbA1c levels (Supplementary Figs. 1C and 2C) and the pancreatic insulin content among corresponding groups (Supplementary Figs. 1D and 2D), respectively.

**Diabetic cardiomyopathy in CB1R−/− mice was characterized by decreased load-dependent +dP/dt; ejection fraction, stroke work, cardiac output and load-independent [Emax dp/dtmax–end-diastolic volume relation, preload-recruitable stroke work] indices of LV systolic contractile function, and impaired diastolic performance (decreased –dP/dt, prolonged time constants of LV pressure decay [τWeiss], increased LV end-diastolic pressure, and decreased slope of the end-diastolic pressure-volume relation [aP/V0] of LV stiffness) function [Fig. 1C and D]. The diabetes-induced cardiac dysfunction was less pronounced in CB1R−/− mice than in CB1R+/+ mice (Fig. 1C and D). There was no difference in the cardiac function in control CB1R−/− and CB1R+/+ mice. The baseline heart rates were similar in both CB1R−/− and CB1R+/+ mice (520 ± 15, n = 9, vs. 529 ± 19, n = 9)
and were decreased to a similar extent after 3 months of diabetes (467 ± 21, n = 9, vs. 452 ± 20, n = 9), respectively.

**Attenuated diabetes-induced myocardial inflammation, oxidative/nitrative stress, β-MHC isozyme switch, and AT1R expression in CB1<sup>-/-</sup> mice.** LV mRNA expression of inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, adhesion molecules (intracellular adhesion molecule [ICAM]-1/vascular cell adhesion molecule [VCAM]-1) (Fig. 2A), iNOS but not endothelial and neuronal nitric oxide synthases (eNOS and nNOS), ..., cyclooxygenase 2 (COX2) (Fig. 2B; Supplementary Fig. 5), AT1R, and p47phox (Fig. 2D), was upregulated in the diabetic myocardium. This result was concordant with the profound oxidative/nitrative stress, characterized by the accumulation of lipid peroxidation product 4-hydroxynonenal and 3-NT (Fig. 2E), enhanced β-MHC isozyme switch
Attenuated diabetes-induced myocardial RAGE and AGE expression/accumulation, MAPK activation, and cell death in CB1−/− mice. Diabetes increased LV RAGE mRNA expression, AGE accumulation (Fig. 3A), p38/JNK MAPK induction (Fig. 3B), enhanced caspase 3, PARP
activation, and DNA fragmentation in CB1+/- diabetic animals (Fig. 3C and D). These changes were attenuated in mice lacking the CB1R.

**Attenuated diabetes-induced myocardial fibrosis in CB1-/- mice.** Diabetes induced marked LV interstitial fibrosis (Fig. 4A) and enhanced mRNA expressions of fibrotic markers (connective tissue growth factor, transforming growth factor-β, fibronectin, and collagen-I) (Fig. 4B) in the CB1+/- mice, which were blunted in diabetic CB1-/- mice (Fig. 4A and B). **CB1R inhibition attenuates diabetes-induced cardiac dysfunction.** Chronic treatment (11 weeks) with CB1R antagonist SR141716/rimonabant (SR1) improved both systolic and diastolic cardiac dysfunction associated with diabetes (Fig. 5A and B). Four weeks of treatment of 8-week diabetic mice with rimonabant also resulted in similar

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**FIG. 3.** Attenuated diabetes-induced myocardial RAGE and AGE expression/accumulation, MAPK activation, and cell death in CB1-/- mice. **A:** LV mRNA expression of RAGE and accumulation of AGES in the myocardium measured by ELISA. **B:** Representative Western immunoblot for the analysis of MAPKs (p38 and JNK) in the myocardial tissues. *P < 0.05 vs. CB1+/- control (CO); #P < 0.05 vs. CB1+/- diabetes (Diab); n = 6/group. C and D: Markers of cell death (PARP and caspase 3 activities and chromatin fragmentation) in the LV myocardial tissues from the respective groups. *P < 0.05 vs. WT/CB1+/- control (CO); #P < 0.05 vs. CB1+/- diabetes (Diab); n = 8/group. (A high-quality color representation of this figure is available in the online issue.)
but less pronounced LV functional improvements (Supplementary Fig. 6).

CB1R inhibition attenuates diabetes-induced myocardial inflammation, oxidative/nitrative stress, β-MHC isozyme switch, AT1R, RAGE and AGE expression/accumulation, p38/JNK MAPK activation, and cell death. Diabetes enhanced LV myocardial inflammation (Fig. 6A and B), oxidative/nitrative stress (Fig. 6B, D, and G), mRNA expression of AT1R, p47phox (Fig. 2D), RAGE, accumulation of AGE (Fig. 2F), β-MHC isozyme switch (Fig. 2C), and decreased SERCA2 mRNA, which were attenuated by chronic treatment (11 weeks) of diabetic animals with CB1R antagonists SR141716/rimonabant (SR1) and AM281 (AM). Chronic treatment also attenuated the diabetes-induced LV

FIG. 4. Attenuation of diabetes-induced myocardial fibrosis in CB1<sup>−/−</sup> mice. A: Representative formalin-fixed paraffin-embedded myocardial tissue sections stained with Sirius Red, indicating the marked interstitial fibrosis in the WT diabetic mice, which was attenuated in CB1<sup>−/−</sup> mice. *P < 0.05 vs. CB1<sup>+/+</sup> control (CO); #P < 0.05 vs. CB1<sup>−/−</sup> diabetes (Diab); n = 6/group. B: mRNA expression of fibrosis markers in the myocardial tissues: *P < 0.05 vs. CB1<sup>+/+</sup> control (CO); #P < 0.05 vs. CB1<sup>−/−</sup> diabetes (Diab); n = 9/group. CTGF, connective tissue growth factor. (A high-quality digital representation of this figure is available in the online issue.)
MAPK activation (Fig. 7A) and apoptotic and PARP-dependent cell death (Fig. 7B and C).

**CB1R inhibition attenuates diabetes-induced myocardial fibrosis.** Diabetes enhanced myocardial fibrosis characterized by increased collagen accumulation (Fig. 8A) and enhanced expression of mRNA markers of fibrosis (Fig. 8B), which were attenuated by chronic treatment (11 weeks) of diabetic animals with CB1R antagonists SR1 and AM.

**DISCUSSION**

The salient findings emanating from the current study are as follows: 1) diabetes leads to upregulation of CB1R expression and increase in endocannabinoid anandamide/AEA levels in the myocardium; 2) diabetes-induced myocardial dysfunction is improved in CB1−/− mice or in diabetic mice treated with CB1 antagonists; 3) genetic deletion or pharmacological inhibition of CB1 receptors attenuates MAPK activation, cell death, inflammation, and oxidative/nitrative stress in diabetic hearts; 4) likewise, it mitigates expression of RAGE, AT1-R p47(phox) NADPH oxidase subunit, and impaired expression of SERCA2a and β-MHC isozyme switch; and 5) the diabetes-induced myocardial accumulation of AGEs and fibrosis are attenuated in CB1−/− mice or in diabetic mice treated with CB1 antagonists.

CB1 receptors are expressed in endothelial (29,37,38), vascular smooth muscle (39,40), and inflammatory cells (30,31) and in cardiomyocytes (26,27,41). It is noteworthy that activation of cardiovascular CB1 receptors by over-produced endocannabinoids has been implicated in the development of pathophysiological alterations and compromised cardiovascular function associated with various forms of shock, cirrhotic cardiomyopathy, and heart failure (21,26–28). There is also increasing recognition that in various pathological conditions, CB1R activation by endocannabinoids may trigger activation of signaling pathways (e.g., p38 and JNK-MAPKs promoting cell death) (21,27,42,43). Recent studies have also demonstrated that CB1R activation by endocannabinoids or synthetic agonists in human coronary artery endothelial cells (29) and in primary human or murine cardiomyocytes (27) triggered increased p38 and JNK activation and ROS generation promoting cell death. CB1R activation also induced ROS generation and TNF-α production in human macrophages that depended on the p38 MAPK pathway and could be attenuated by its inhibition (30), p38 MAPK inhibition also attenuated CB1-mediated cell death in endothelial cells and cardiomyocytes (27,29). Consistently with the above-mentioned studies in preclinical models of heart failure (26–28), atherosclerosis (31,44), and diabetic retinopathy...
CB1 deletion or pharmacological inhibition limits the vascular or myocardial inflammation and/or oxidative/nitrative stress and interrelated cell death and disease progression. CB1 antagonists also exerted numerous unexpected beneficial effects (e.g., anti-inflammatory effects) in clinical trials of obesity beyond their effects on body weight (23,25,35,45), and peripheral CB1R blockade appears to be a promising approach in the treatment of visceral obesity and its cardiometabolic complications (45,46).

It is noteworthy that increased plasma endocannabinoid levels positively correlate with coronary circulatory dysfunction in human obesity (32). We found increased CB1R expression and endocannabinoid anandamide levels in the left ventricle of diabetic hearts, which is consistent with elevated anandamide levels reported in the retina of patients with diabetic retinopathy (47). Increased endocannabinoid levels have also been reported in serum of patients with type 2 diabetes and their subcutaneous tissue (35). Although the mechanism of marked upregulation of

FIG. 6. Attenuation of diabetes-induced myocardial inflammation, oxidative/nitrative stress, β-MHC isozyme switch, and AT1R expression by CB1R antagonists. A: LV mRNA expressions of inflammatory cytokines and adhesion molecules. B: iNOS and COX2. C: α- and β-MHC. D: AT1R, p47phox, gp91phox, and NADPH isoforms. E: SERCA2a. F: mRNA expression of RAGE and accumulation of AGE in the myocardium measured by ELISA. G: Oxidative/nitrative stress determined by measuring 4-HNE and 3-NT in the LV myocardial tissues in the respective groups as indicated. *P < 0.05 vs. vehicle control (CO); #P < 0.05 vs. diabetes (Diab), n = 8–9/group. AM/SR1 treatments alone in control mice had no significant effect on any of the markers studied (not shown) compared with vehicle-treated controls (CO). (A high-quality color representation of this figure is available in the online issue.)
CB₁ receptors in various peripheral tissues during multiple disease conditions associated with increased inflammation and/or oxidative stress has not been evaluated in much detail (21,45), this process most likely may involve activation of ROS- and/or inflammation-dependent transcription factors. Indeed, in rat mesangial cells, high glucose upregulates CB₁ mRNA expression in an NF-κB–dependent manner. Similarly, hyperglycemia-induced upregulation of CB₁ has also been reported in retina pigment epithelial cells recently (rev. in 35). CB₁ antagonists attenuated the high glucose–induced apoptosis in mesangial, retina pigment, and endothelial cells (35). Reactive oxygen and nitrogen species and inflammatory mediators such as TNF-α, which are also known to be triggered/upregulated by hyperglycemia, have been implicated in enhanced endocannabinoid production through the activation of NF-κB and other pathways in several cell types (including monocytes/macrophages and cardiomyocytes), as well as by inactivation and/or downregulation of the endocannabinoid-metabolizing enzyme fatty acid amide hydrolase. Consistently, hyperglycemia induced fatty acid amide hydrolase– and CB₁–dependent cell death in retina pigment epithelial cells (rev. in 35). Notably, fatty acid amidase knockout mice, which have approximately two- to threefold increased myocardial anandamide levels, have markedly increased mortality, cardiac dysfunction, and myocardial cell death in acute and chronic heart failure models, which are attenuated by CB₁ antagonists (28). More importantly, elevated plasma endocannabinoid levels show very strong positive correlation with coronary circulatory dysfunction and adverse cardiac events in obese human subjects (32).

Growing evidence implies that oxidative/nitrative stress together with activation of various proinflammatory and cell death pathways play pivotal roles in the development of complex biochemical, mechanical, and structural alterations associated with diabetic cardiomyopathy (3–6,10,14–16). Unfortunately, despite the accumulating knowledge obtained during the past decades, the treatment of diabetic cardiomyopathy is poor and largely symptomatic (1).

In the current study, using a well-characterized mouse model of type 1 diabetic cardiomyopathy (3,5,14,16), we evaluated the effects of genetic deletion or pharmacological inhibition of CB₁ receptors with selective CB₁ antagonists (for 11 weeks administered after the destruction of pancreatic β-cells and development of frank type 1 diabetes) on myocardial dysfunction, inflammation, oxidative/nitrative stress, cell death, fibrosis, and interrelated signaling.
pathways. We also evaluated the effect of CB1 inhibition on cardiac dysfunction associated with already-established diabetic cardiomyopathy (4 weeks' treatment of 8-week diabetic mice).

Consistent with previous reports (3,5–7,13–16), diabetic cardiomyopathy was characterized by declined diastolic and systolic myocardial performance, increased oxidative/nitrative stress (4-HNE, 3-NT, iNOS), activation of various stress signaling pathways (e.g., JNK and p38 MAPK), enhanced expression of RAGE and AT1R, p47(phox) NADPH oxidase subunit, accumulation of AGEs, inflammation (increased expression of TNF-α, IL-1β, COX2, adhesion molecules ICAM-1 and VCAM-1), β-MHC isoyme switch, myocardial fibrosis, and decreased expression of SERCA2a.

Compelling evidence (both from rodent models of STZ-induced type 1 diabetes and human myocardial biopsies) suggests that the renin-angiotensin system is upregulated with diabetes and angiotensin II locally through AT1R and is overexpressed in diabetic hearts or in cardiomyocytes exposed to high glucose, contributing to the development of diabetic cardiomyopathy (3,10–13). The beneficial effects of AT1R blockade in diabetic hearts involve, but are not limited to, the attenuation of myocardial NADPH oxidase-dependent (such as p47phox) ROS generation, inflammation, cell death, fibrosis, and contractile dysfunction (3,10–13). Xanthine oxidase, cyclooxygenase, mitochondrial electron transport chain, activated inflammatory cells, and uncoupled endothelial nitric oxide synthase may also

FIG. 8. Attenuation of diabetes-induced myocardial fibrosis by CB1 antagonists. A: The representative formalin-fixed paraffin-embedded myocardial tissue sections stained with Sirius Red, indicating the marked fibrosis in the diabetic mice, which was attenuated by CB1R antagonists. *P < 0.05 vs. vehicle/AM/SR1 control (CO); #P < 0.05 vs. diabetes (Diab), n = 6/group. B: mRNA expression of fibrosis markers in the myocardial tissues. *P < 0.05 vs. vehicle/AM/SR1 control (CO); #P < 0.05 vs. diabetes (Diab), n = 9/group. (A high-quality digital representation of this figure is available in the online issue.)
represent additional sources of ROS generation in diabetic hearts (4). Convincing in vitro, ex vivo, and in vivo evidence suggests that high glucose–induced increased iNOS expression contributes to cardiac dysfunction associated with type 1 diabetic cardiomyopathy via formation of reactive nitrogen species such as peroxynitrite through the rapid diffusion-limited reaction of superoxide anion (derived from NADPH oxidase and other sources) and nitric oxide derived from iNOS (rev. in 4). The above-mentioned reaction is faster than the decomposition of the superoxide by superoxide dismutase; therefore, it results in loss of the beneficial effects of nitric oxide (it is immediately converted to reactive nitrogen species in the presence of superoxide before is able to exert its known protective effects) (4). High glucose–induced ROS/reactive nitrogen species also induces modifications of important proteins involved in Ca2+ handling and myocardial contractility (e.g., SERCA2a) (4) and decrease of their expression/function (18–20), lipid peroxidation, oxidative DNA damage, activation of stress signaling, and other cell death pathways, among others (4). p38 MAPK activation appears to play an important role in pathogenesis of diabetic cardiomyopathy, since its pharmacological inhibition attenuates not only myocardial dysfunction, but also the expression of cardiac inflammatory markers, such as TNF-α, interleukin-1β, interleukin-6, and myocardial fibrosis (15).

Hyperglycemia and/or hyperglycemia-induced ROS (also involving enhanced AT1R expression/signaling) may lead to increased accumulation of products of nonenzymatic glycation/oxidation of proteins/lipids (AGE) and enhanced expression of their receptor (RAGE) in the vasculature and myocardium; these products are thought to play a key role in the development and progression of cardiovascular complications of diabetes (7,8). In hearts of type 1 diabetic rodents, increased expression of RAGE and accumulation of AGEs have been associated with diabetes-induced dysfunction and structural alterations (7,8). In diabetic heart failure patients with reduced LV ejection fraction, the fibrosis and accumulation of AGEs contribute to the increased diastolic stiffness, but in patients with normal LV ejection fraction, the increased cardiomyocyte resting tension is responsible for this phenomenon (9).

Genetic deletion of CB1 receptors or its pharmacological inhibition with selective CB1 antagonists attenuated the diabetes-induced myocardial dysfunction, expression of AT1R and RAGE, accumulation of AGEs, oxidative/nitrative stress and inflammation [4-HNE, 3-NT, iNOS, p47(phox), TNF-α, IL-1β, COX2, ICAM-1, and VCAM-1], activation of myocardial p38/JNK MAPKs, cell death, and fibrosis and also restored the impaired expression of SERCA2a and MHC isozyme switch.

Interestingly, in recent provocative studies, Hunyady’s group (rev. in 48) proposed a paracrine transactivation of the CB1 cannabinoid receptor by AT1 and other Gq/11 protein–coupled receptors, implying that this signaling may overlap in a pathological situation. Indeed, a recent study demonstrated that CB1R and AT1R functionally interact forming heteromers, resulting in the potentiation of AT1R signaling (49). AT1R–CB1R heteromers and enhancement of angiotensin II–mediated signaling were demonstrated in hepatic stellate cells from ethanol–administered rats (in which CB1R was upregulated), and CB1 inhibition prevented the angiotensin II–mediated mitogenic signaling and profibrogenic gene expression (49) underlying the functional significance of this interaction. A recent study also described that chronic CB1R inhibition led to decreased vascular AT1R expression, NADPH oxidase–derived vascular oxidative stress, and improved endothelial function in apolipoprotein E–deficient mice fed a cholesterol-rich diet (40). In cultured vascular smooth muscle cell, CB1 antagonists reduced angiotensin II–mediated NADPH oxidase-dependent ROS generation and downregulated AT1R expression, whereas CB2R agonist upregulated AT1R, indicating that AT1R expression is directly regulated by the CB2R. In light of the above-mentioned observations, our current study demonstrating decreased AT1R and p47(phox) expression in hearts of CB1 knockout diabetic mice or mice treated with CB1 antagonists also suggests an important interaction of CB1 and AT1R signaling. The downregulation of the AT1R-NADPH oxidase–ROS pathway by CB2R inhibition/genetic deletion could also contribute to decreased oxidative stress, AGE/RAGE accumulation/signaling, MAPK activation, cell death, and fibrosis observed under these conditions, in addition to the direct inhibitory effect of the overproduced endocannabinoid-CB1 signaling on MAPK activation and its multiple above-discussed consequences in cardiovascular cell types. The attenuated myocardial fibrosis and AGE accumulation in hearts of the diabetic CB2R knockout mice or mice treated with SR141716/rimonabant is most likely responsible for the decreased diastolic stiffness observed.

It is noteworthy that our results also suggest that CB1 inhibition may preserve its beneficial effects on contractile dysfunction even if administered after the development of established cardiomyopathy. This result coupled with recent studies demonstrating that CB2R blockade and/or its genetic deletion attenuates proteinuria and/or vascular inflammation and cell death in experimental models of type 1 diabetic nephropathy (33), retinopathy (34), and neuropathy (35), and increases pancreatic β-cell proliferation and mass before the complete destruction of these cells in early diabetes (50), are very exciting from a therapeutic point of view.

Collectively, our results strongly suggest that overactivation of endocannabinoid system and CB1 receptors may play an important role in the pathogenesis of diabetic cardiomyopathy by facilitating AT1R expression/signaling, RAGE and AGE expression/accumulation/signaling, MAPK activation, oxidative/nitrative stress, inflammation, cell death, fibrosis, and contractile dysfunction. Conversely, CB1 inhibition may be of significant benefit in the treatment of diabetic cardiovascular complications and possibly other complications.

ACKNOWLEDGMENTS

This study was supported by the Intramural Research Program of NIH/NIAAA (to P.P.). B.H. was supported by an Alexander von Humboldt Foundation and the European Union 7th Framework fellowship (MB08-A80238). S.B. was supported by the Hungarian National Research Fund–European Union 7th Framework (FP7-CIG-294278).

No potential conflicts of interest relevant to this article were reported.

M.R. researched data and reviewed, edited, and wrote the manuscript. S.B., M.K., P.M., W.S.L., B.H., E.H., R.C., L.L., and G.H. researched data and contributed to discussion. K.M. contributed new reagents, edited the manuscript, and contributed to discussion. P.P. wrote the manuscript, reviewed and edited the manuscript, and contributed to discussion. P.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
The authors are indebted to Judith Harvey-White (NIH/NIAAA) and Dr. George Kunos (NIH/NIAAA) for endocannabinoid measurements and Dr. Kunos for providing support and resources for the completion of this study.

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