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Review

The Endocannabinoid System and Plant-Derived Cannabinoids in Diabetes and Diabetic Complications

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Oxidative stress and inflammation play critical roles in the development of diabetes and its complications. Recent studies provided compelling evidence that the newly discovered lipid signaling system (ie, the endocannabinoid system) may significantly influence reactive oxygen species production, inflammation, and subsequent tissue injury, in addition to its well-known metabolic effects and functions. The modulation of the activity of this system holds tremendous therapeutic potential in a wide range of diseases, ranging from cancer, pain, neurodegenerative, and cardiovascular diseases to obesity and metabolic syndrome, diabetes, and diabetic complications. This review focuses on the role of the endocannabinoid system in primary diabetes and its effects on various diabetic complications, such as diabetic cardiovascular dysfunction, nephropathy, retinopathy, and neuropathy, particularly highlighting the mechanisms beyond the metabolic consequences of the activation of the endocannabinoid system. The therapeutic potential of targeting the endocannabinoid system and certain plant-derived cannabinoids, such as cannabidiol and Δ9-tetrahydrocannabivarin, which are devoid of psychotropic effects and possess potent anti-inflammatory and/or antioxidant properties, in diabetes and diabetic complications is also discussed. ***(Am J Pathol 2012, 180:432–442; DOI: 10.1016/j.ajpath.2011.11.003)***

Endocannabinoids (ECs) are endogenous, bioactive lipid mediators that exert their effects mainly through specific G protein–coupled (primarily Gi/o) receptors: cannabinoid-1 (CB_1) receptor and cannabinoid-2 (CB_2) receptor. The signaling of these receptors is complex and, depending on the cell type, may involve inhibition (also activation in certain cases) of adenyl cyclase activity, activation of various mitogen-activated protein kinases (MAPKs) (eg, p38- and p44/42-MAPKs, c-Jun N-terminal kinase, and extracellular signal–regulated kinase), protein kinases A and C, and modulation of various Ca^{2+} and K^+ channels.[1–3] Previously, it was thought that the CB_1 receptor was predominantly expressed in the central nervous system, mediating undesirable psychoactive effects, whereas the CB_2 receptor was expressed mainly in immune and hematopoietic cells, modulating immune activities. However, recent studies also have demonstrated the expression of these receptors in various other cell types, both centrally and in the peripheral organs, implicating these receptors in a wide range of physiologic and pathologic functions and activities.[1,4,5] In addition to their primary target cannabinoid receptors, ECs and possibly their metabolites may also activate multiple receptor-dependent and -independent mechanisms.[3]

The two main ECs are anandamide (AEA) and 2-arachidonoyl glycerol (2-AG).[6,7] They are synthesized "on demand," not stored in the cell, and are degraded quickly to have a transient and localized effect.[5] Their synthesis is mainly dependent on intracellular Ca^{2+} concentrations because AEA is mainly formed via a two-step pathway composed of a Ca^{2+}-dependent N-acyltransferase and N-acylphosphatidylethanolamine-hydrolyzing phospholipase D, whereas diacylglycerol lipase and phospho-

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lipase C-β are mainly responsible for the synthesis of 2-AG. Their main metabolizing enzymes are fatty acid hydrolase (FAAH) and monoacylglycerol lipase (MAGL), the former favoring AEA and the latter favoring 2-AG catabolism.[8] AEA and 2-AG bind to both the CB_1 and CB_2 receptors; however, AEA binds with higher affinity to the CB_1 receptor, whereas 2-AG favors the CB_2 receptor.[1] Because of rapid degradation, ECs and their metabolites may also exert multiple, important biological effects unrelated to the activation of conventional cannabinoid receptors.[1–3] The ECs, their specific receptors, and the synthesizing and metabolizing enzymes form the EC system (ECS).[5,8] The modulation of the ECS has therapeutic potential in a wide range of disparate diseases and pathologic conditions that affect humans, including neurodegenerative, kidney, and gastrointestinal diseases, pain, cancer, bone and cardiovascular disorders, obesity and metabolic syndrome, and inflammation, just to mention a few.[1,4,5]

The cannabinoid receptors are, at least in part, also responsible for the effects of several natural constituents of *Cannabis sativa* (ie, the marijuana plant).[3,9] The most characterized plant-derived cannabinoid, Δ9-tetrahydrocannabinol (THC), was previously considered the only active ingredient of marijuana, responsible for its undesirable psychotropic effects mediated by central CB_1 receptors, greatly limiting its potential therapeutic use. Numerous recent studies have also focused on two natural plant-derived constituents with very negligible psychotropic effects and great therapeutic potential in inflammatory diseases, diabetes, and diabetic complications: cannabidiol (CBD) and Δ9-tetrahydrocannabivarin (THCV).[3,9] CBD is the most abundant nonpsychotropic constituent of *C. sativa* and has been reported to exert protective effects in multiple disease models,[9] including diabetes[10,11] and diabetic complications.[12–15] CBD is well tolerated without adverse effects when administered in the long term to humans and has been approved for the treatment of inflammation, pain, and spasticity associated with multiple sclerosis in Canada, the United Kingdom, and Spain. THCV seems to be a promising therapeutic compound because it has been shown to behave as a CB_1 receptor antagonist; at the same time, it activates CB_2 receptors, thereby decreasing inflammation and oxidative stress,[16,17] which are key processes in the development of diabetes and diabetic complications.

Diabetes mellitus affects 8.3% of the US population and is the seventh leading cause of death in the United States.[18] Type 1 diabetes mellitus (insulin-dependent or juvenile onset) commonly has an increased prevalence of autoantibodies against pancreatic islet cells, which are thought to play an important role in the destruction of insulin-producing β-cells. This type of diabetes is usually diagnosed in individuals younger than 30 years and has a prevalence of 0.2% to 0.5%. Patients have a lean body build and are prone to ketosis, owing to absent insulin production. Type 2 diabetes (non–insulin-dependent or maturity onset) is often characterized by a combination of a progressive insulin secretory defect in pancreatic β-cells and resistance to the effects of insulin in peripheral target tissues. This type of diabetes has a prevalence of 2% to 4% and is more common in men. Patients are usually older than 40 years and obese. Both types of diabetes are characterized by high blood glucose levels (hyperglycemia) and consequent metabolic alterations, which eventually lead to the development of multiple complications.

Most diabetic complications are associated with pathologic alterations in the vascular wall; the most common macrovascular complication of diabetes is atherosclerosis, which increases the risk of myocardial infarction, stroke, and peripheral artery disease, whereas microvascular complications underlie nephropathy, retinopathy, and peripheral neuropathy.[19] Diabetic complications have tremendous physical, emotional, and economic impact because diabetes is the leading cause of kidney failure, nontraumatic lower-limb amputation, and new cases of blindness among adults in the United States.[18] Hyperglycemia, caused by either a lack of insulin or insulin resistance, triggers tissue damage via a multiple complex mechanism, leading to the accumulation of sorbitol and advanced glycation end products, while increasing the expression of its receptor. Hyperglycemia also activates protein kinase C and the hexosamine pathway. Several studies have indicated that the common upstream event in the pathogenesis of diabetic complications is the formation of reactive oxygen species (ROS) and reactive nitrogen species.[20,21] We provide a brief overview of the role of the ECS in the pathogenesis of diabetes and diabetic complications and the therapeutic potential of the modulation of this endogenous system and certain natural (plant-derived) cannabinoids with antioxidant and anti-inflammatory properties.

Role of the ECS in Diabetes and Diabetic Complications

Primary Diabetes

Diabetes is characterized by hyperglycemia caused by either a lack of insulin (due to autoimmune destruction of islet cells) or insulin resistance. Obesity is the main risk factor for type 2 diabetes, leading to insulin resistance. Exogenous cannabinoids and ECs increase food intake and promote weight gain in animals by activating central CB_1 receptors.[22] Furthermore, activation of the peripheral ECS has been observed in human obesity,[23] leading to adipogenesis, lipogenesis, hepatic steatosis, and increased insulin resistance, most likely involving both peripheral and central CB_1 receptors.[24,25] Blockade of CB_1 receptors with rimonabant (RIO) has been promising in clinical trials, leading to weight loss and improvements in several metabolic risk factors (eg, decreased waist circumference, increased high-density lipoprotein cholesterol levels, and decreased triglyceride levels) that cannot be explained by the observed weight loss.[26–28] The CRESCENDO trial, testing RIO for the prevention of cardiovascular events, however, was abruptly terminated because of the drug's neuropsychiatric adverse effects.[29] However, the fact that a peripheral CB_1 receptor antagonist was also able to efficiently reduce weight and improve metabolic risk factors in a mouse model of obe-

sity[30] gave hope that modulation of the ECS might still be a viable option to tackle human obesity.[25] Indeed, there is considerable interest in the development of peripheral CB_1 receptor antagonists.[25]

The presence and function of the ECS in islet cells have been intensively investigated. The results regarding the expression of cannabinoid receptors have been contradictory and show a strong species dependence. In mouse islet cells, both CB_1 and CB_2 receptors are expressed[31–34]; however, the specific cell type that expresses these receptors is still under debate. It has also been shown that EC-synthesizing enzymes are also present in α-cells, whereas the metabolizing enzymes are restricted to β-cells[33,35] with questionable expression of MAGL in α-cells.[35] There is consensus that CB_1 receptors are expressed in rat pancreatic islets; however, the presence of CB_2 receptors is debated.[32,36,37] MAGL is expressed in δ-cells, and FAAH is expressed in α-cells.[36] More importantly, in the human pancreas, CB_1 receptor, MAGL, and FAAH expression has been confirmed, but there is no agreement about either their localization or CB_2 receptor expression.[35,36,38] Although there is a lot of controversy, it seems that most studies agree that CB_1 receptors are expressed in islet cells; recently, CB_1 receptor also has been implicated in insulin secretion. A CB_1 receptor agonist was shown to increase insulin secretion in RINm5F cells,[39] MIN6 cells,[40] rat islet cells,[37] and mouse islet cells.[34] Similar findings were confirmed in human islets cells.[38] There is only one report that showed that AEA and a CB_1 agonist decreased insulin production.[41] Results regarding the role of the CB_2 receptor in insulin secretion are also contradictory. Although one group found an increase in insulin release on CB_2 receptor activation,[34,40] others have shown its attenuation.[31,38] CB_1 receptor inhibition has recently been reported to increase β-cell proliferation, which is exciting from a therapeutic point of view.[35]

It seems that the debate has not yet settled about the exact role of cannabinoids in pancreatic islet cells, and the conflicting results might be attributable to the different species and experimental conditions used in these studies. The most important fact is, however, that clinical trials are sending a clear message about the role of the ECS in the pathogenesis of primary diabetes. The first clinical trial (RIO Diabetes) aimed to clarify the efficacy and safety of the CB_1 antagonist RIO in obese or overweight patients with type 2 diabetes inadequately controlled by either metformin or sulfonylureas.[42] Patients receiving RIO treatment showed greater weight loss, reduction in waist circumference, hemoglobin A_{1c} levels, and fasting glucose concentrations compared with placebo. There was also a significant improvement in high-density lipoprotein cholesterol, triglyceride, and non–high-density lipoprotein cholesterol levels, as well as in systolic blood pressure. In drug-naive patients with type 2 diabetes, RIO showed a similar efficacy and caused significant improvements in glycemic control, body weight, and metabolic profile (SERENADE trial).[43] Recently, an interesting study investigated the effects of CB_1 antagonist therapy in insulin-treated patients with type 2 diabetes (ARPEGGIO trial),[44] and the addition of RIO to the patients' standard insulin treatment improved glycemic control and cardiometabolic risk factors.

The pivotal role of the ECS in the pathogenesis of diabetes was further supported by elevated EC levels in diabetic patients. Patients with type 2 diabetes had higher serum levels of both AEA and 2-AG than did healthy volunteers,[39] and AEA levels were also increased in the subcutaneous tissues of these individuals.[45]

There is also considerable interest in the use of certain natural and similar synthetic cannabinoid ligands to modulate a wide variety of immune responses, including T-lymphocyte activation and subsequent cytokine production.[17,46] THC was shown to attenuate the severity of autoimmune responses in an experimental model of autoimmune diabetes as evidenced by the significantly lower number of infiltrating lymphocytic cells and reduced expression levels of interferon-γ, interleukin-12, and tumor necrosis factor-α (TNF-α).[47] The treatment also preserved pancreatic insulin content and led to lower blood glucose levels compared with the untreated diabetic group. Even though THC shows excellent immunosuppressive ability, the psychoactive effects of the compound limit its usefulness for therapeutic purposes. This is the reason why the study[10] that showed that CBD exerts similar beneficial effects is crucially important. CBD reduced the incidence of diabetes in nonobese diabetic mice, the mouse model of type 1 diabetes. The effect was paired with reduced insulitis, which was due to a shift of the immune response from Th1 to Th2 dominance, resulting in decreased levels of proinflammatory cytokines, such as interferon-γ and TNF-α. CBD was also able to ameliorate the disease when given at the time of the development of initial symptoms of diabetes in nonobese diabetic mice.[11]

Collectively, even though the ECS seems to play an important role in the development and control of primary diabetes, the exact mechanisms and cellular targets are still not completely understood. In the near future, the role of cannabinoid receptors in the regulation of islet cell function must be further investigated, and it is important to develop a peripheral CB_1 receptor antagonist suitable for clinical trials. Plant-derived cannabinoids, which are not toxic to humans and devoid of psychoactive effects, such as cannabidiol, may represent a promising new avenue to target autoimmune diabetes and protect pancreatic β-cells from oxidative injury.

Cardiovascular Complications

Accurate glucose, blood pressure, and plasma lipid controls, as well as preventive care practices, are effective in reducing the number of complications in certain patient cohorts with diabetes; however, they have their own limitations. For example, although intensive glucose-lowering therapy reduces glycated hemoglobin levels, it increases 5-year mortality compared with standard therapy (ACCORD trial).[48] The understanding of the pathogeneses of microvascular and macrovascular complications in diabetes is paramount for the development of new therapeutic targets. Recently, several studies highlighted the important role of the ECS in the regulation of vascular

inflammation, oxidative stress, and atherosclerosis,[49] suggesting that the modulation of the ECS and the administration of plant-derived cannabinoids with antioxidant and anti-inflammatory properties might be beneficial in the treatment of cardiovascular complications associated with diabetes.

Both CB_1 and CB_2 receptors are expressed in the cells of the cardiovascular system, including cardiomyocytes, fibroblasts, endothelial and vascular smooth muscle cells, and infiltrating immune cells.[49] CB_1 receptor activation by AEA or synthetic agonists induces ROS production, MAPK activation, and cell death in human coronary endothelial cells,[50] mimicking the pathogenesis of diabetes-induced endothelial dysfunction.[21] The activation of MAPK is only partially mediated by ROS, suggesting direct CB_1 receptor–mediated MAPK activation. Furthermore, activation of CB_1 receptors leads to increased angiotensin-1 receptor expression and nicotinamide adenine dinucleotide phosphate oxidase activity, which contribute to ROS production.[51] In contrast to CB_1 receptor activation, the activation of the CB_2 receptor is coupled with decreased endothelial cell activation, monocyte-endothelial adhesion, and transendothelial monocyte migration after TNF-α or lipopolysaccharide stimulation,[52] hallmarks of the development of atherosclerosis. Activation of the CB_2 receptor also attenuates TNF-α–triggered activation of both NF-κB and Rho, up-regulation of adhesion molecules, and increased expression levels of monocyte chemoattractant protein-1 (MCP-1) in endothelial cells. CB_1 receptor activation by ECs and/or synthetic ligands also promotes ROS generation, MAPK activation, and inflammatory responses in macrophages and neutrophils,[53,54] whereas CB_2 receptor activation exerts opposing functions.[4]

Vascular smooth muscle proliferation and migration are also key events in the pathogenesis of atherosclerosis and, therefore, in all macrovascular complications of diabetes. CB_1 receptors were suggested to play an important role in this event because receptor blockade was able to inhibit vascular smooth muscle proliferation and migration in response to platelet-derived growth factor stimulation by inhibiting Ras and extracellular signal–regulated kinase 1/2 activation.[55] The application of a CB_2 receptor agonist showed a similar efficacy in the attenuation of vascular smooth muscle proliferation,[56] indicating an opposing role of the cannabinoid receptors in both endothelial cell activation and vascular smooth muscle proliferation.

The first direct evidence that cannabinoid receptors play a key role in the pathogenesis of atherosclerosis came from an *ApoE*$^{-/-}$ mouse model.[57] THC treatment reduced the development of atherosclerotic plaques and macrophage content through the activation of CB_2 receptors. The antiatherosclerotic properties of THC were associated with a reduction of the T_H1 response and an inhibition of monocyte/macrophage migration to the site of inflammation. Later, it was shown that the CB_1 receptor antagonist RIO was also able to inhibit atherosclerosis in mouse models.[58,59] Its beneficial effects paralleled an improved metabolic profile and a decreased inflammatory cytokine level, decreasing thioglycollate-induced macrophage recruitment.

The relevance of these described findings in metabolic syndrome was investigated by long-term RIO treatment in obese Zucker rats.[60] RIO was able to attenuate increased systolic blood pressure and metabolic abnormalities without altering endothelium-dependent relaxation and restored vascular contraction induced by α-adrenergic agonists. RIO also increased cyclooxygenase 2 expression and prostacyclin production in the aortas of obese Zucker rats.[61] In a rat model of prediabetic metabolic syndrome, long-term RIO treatment did not alter macrovascular response, as shown by the unaltered endothelial function of aortic rings and the incidence of ischemic myocardial lesions, but it diminished microvascular complications, reducing the albumin-creatinine ratio, an index of renal vascular function, and the fraction of sclerotic glomeruli.[62]

The first clinical trial investigating the potential benefit of long-term CB_1 receptor blockade with RIO on the progression of atherosclerosis in obese patients with coronary artery disease did not have a clear conclusion (STRADIVARIUS trial).[63] Although RIO failed to alter disease progression for the primary end point (ie, atheroma volume), it showed a favorable effect on the secondary end point (ie, total atheroma volume). Additional post hoc exploratory analyses revealed that the changes in mean maximum atheroma thickness were favorably affected by RIO. However, changes in atheroma volume in the most diseased 10-mm subsegments showed no significant difference between treatments. To clarify whether this secondary end point result can be translated into a clinical benefit (eg, myocardial infarction, stroke, and cardiovascular death reduction), the CRESCENDO trial was launched.[29] The trial, however, was prematurely terminated because of increasing concerns related to increased anxiety and suicide rates in the RIO treatment group. Additional trials are needed to clarify whether modification of the ECS can lead to a clinically relevant decrease in macrovascular complications of diabetes, as soon as an effective peripheral CB_1 receptor antagonist[30] or a CB_2 receptor agonist[4] reaches the clinical phase of development.

Independent from macrovascular complications, diabetic cardiomyopathy is a distinct primary disease process that leads to heart failure in diabetic patients. Diabetic cardiomyopathy is characterized by left ventricular hypertrophy and diastolic dysfunction due to myocardial collagen and advanced glycation end product deposition.[64] ROS have been implicated in all stages of the development of heart failure, including cardiac hypertrophy, fibrosis, and contractile dysfunction.[65] The role of the ECS in diabetic cardiomyopathy has not been investigated in detail, even though several studies have shown the involvement of cannabinoid receptors in oxidative stress–related cardiac dysfunction. CB_1 receptors can mediate oxidative stress and cell death in doxorubicin-induced cardiomyopathy models and in human cardiomyocytes[66,67]; this damage is enhanced in mice deficient in the main EC, AEA-metabolizing enzyme, FAAH.[54] CB_1 receptor inhibition by RIO was also shown to be protec-

tive in a myocardial infarction model in which mice were fed a standard or high-fat diet.[68] In contrast, the activation of CB_2 receptors showed cardioprotective effects,[69] which were mediated by three different mechanisms: reduced superoxide production, increased levels of extracellular signal–regulated kinase 1/2 and signal transducer and activator of transcription-3 phosphorylation, and inhibited neutrophil recruitment. Although direct involvement of the ECS has not yet been proven in diabetic cardiomyopathy, the plant-derived cannabinoid CBD attenuates inflammation, oxidative stress, cell death, myocardial dysfunction, and fibrosis in a diabetic cardiomyopathy model.[14] These beneficial effects involve the attenuation of diabetes-induced myocardial NF-κB and MAPK activation and the promotion of survival mechanisms (eg, AKT/protein kinase B activation).

Diabetic Nephropathy

Diabetes is a leading cause of renal failure, accounting for 44% of all new cases in 2008.[18] Hyperglycemia stimulates ROS generation, which ultimately leads (via diverse pathways) to diabetic nephropathy characterized by mesangial expansion, thickening of the glomerular basement membrane, and glomerular sclerosis.[70] There is strong evidence that both the synthetic and degradative pathways of the ECS are present in the kidney,[71] and the CB_1 receptor is expressed in both glomeruli and tubular epithelial cells.[72] In intrarenal arteries, the CB_1 receptor is present in the endothelium,[72] and the CB_2 receptor is present in mesangial cells.[71] Cannabinoid receptors play opposing roles in the regulation of oxidative stress in the kidney, as observed in a murine nephropathy model induced by cisplatin. The CB_1 receptor promotes inflammation, oxidative/nitrative stress, and cell death through the activation of the p38-MAPK pathway.[73] In contrast, CB_2 receptor agonists limit damage after cisplatin administration by reducing oxidative stress, inflammation, and apoptosis.[74] For therapeutic purposes, it is important that plant-derived CBD is also able to ameliorate cisplatin-induced nephrotoxicity.[75]

The first direct indication that the ECS plays an important role in the pathogenesis of diabetic nephropathy came from a murine model of metabolic syndrome.[72] Blockade of CB_1 receptors by RIO prolonged the lifespan of obese Zucker rats, even at a dose that did not influence the development of obesity. This effect was concurrent with a delay in the progression of renal failure as shown by the prevention of the development of proteinuria, improved creatinine clearance, and reduction of glomerular injury and renal hypertrophy compared with vehicle-treated rats. Similarly, RIO was also able to reduce the albumin-creatinine ratio and glomerular sclerosis in a prediabetic rat model of metabolic syndrome.[62] Definitive proof for the direct involvement of CB_1 receptors in diabetic nephropathy arose from a type 1 diabetic model in which metabolic effects did not confound the outcome.[76] The CB_1 receptor was found to be overexpressed by glomerular podocytes after streptozotocin treatment. The selective CB_1 antagonist AM-251 reduced proteinuria by preventing a decrease in the mRNA and protein levels of the slit diaphragm molecules nephrin, podocin, and zonula occludens-1 in diabetic kidneys.[76] Similar to the cisplatin-induced nephropathy model, an opposing protective effect of CB_2 receptor activation was demonstrated in the type 1 diabetic nephropathy model. CB_2 agonists ameliorated albuminuria, podocyte protein down-regulation, and glomerular monocyte infiltration without affecting early markers of fibrosis and reduced chemokine receptor-2 expression in both the renal cortex and cultured podocytes, suggesting that CB_2 receptor activation may interfere with the deleterious effects of MCP-1 signaling.[77] Podocytes express the CB_2 receptor both *in vitro* and *in vivo*. The CB_2 receptor was down-regulated in kidney biopsy specimens from patients with advanced diabetic nephropathy, and renal levels of the CB_2 ligand 2-AG were reduced in diabetic mice, suggesting impaired CB_2 signaling.[77]

The *in vivo* results were supported by *in vitro* findings that provided more mechanistic insight as to how the ECS influences the pathogenesis of renal failure in diabetes and the role of tubular processes in the effects of ECs during the development of diabetic kidney damage. *In vitro*, AEA significantly increases the hypertrophy of proximal tubular cells.[78] CB_1 antagonists reduced and CB_2 antagonists increased the observed hypertrophy. In another study, the hyperlipidemia-induced tubular cell dysfunction observed in diabetic kidneys was modeled by palmitic acid–induced apoptosis in HK-2 cells.[79] In this system, CB_1 receptor overexpression was observed in a cyclooxygenase-dependent manner. Blockade of CB_1 receptors was able to ameliorate palmitic acid–induced endoplasmic reticulum stress and the subsequent apoptosis. In rat mesangial cells, high glucose levels up-regulate CB_1 mRNA expression levels and internalization in NF-κB– and cytosolic phospholipase A_2–dependent manners.[80] CB_1 antagonism prevented high glucose concentration–induced apoptosis via the attenuation of endoplasmic reticulum stress, providing further evidence of the potential beneficial effects of CB_1 receptor blockade in diabetic nephropathy.

Diabetic Retinopathy

Diabetes is the leading cause of new cases of blindness and preventable blindness among adults. Vascular inflammation and endothelial cell death caused by oxidative and nitrative stress are characteristics of diabetic retinopathy.[21] In the early stages, retinopathy is characterized by microaneurysm formation and microvascular lesions and later by extensive intraretinal hemorrhage that culminates in proliferative diabetic retinopathy with neovascularization and either preretinal or vitreous hemorrhage.[81]

The ECS is present in the retina as shown by the presence of AEA, 2-AG, and the metabolizing enzymes FAAH and MAGL.[82] CB_1 receptors are expressed in the layers of the retina, ciliary body, iris, and choroid, whereas CB_2 receptors are localized to the retina.[83] It has been shown that EC levels are elevated in the eyes of patients with diabetic retinopathy.[84] 2-AG levels are elevated in the iris, whereas AEA levels are increased in the

cornea, ciliary body, retina, and the choroid. The role of such an increase gained importance when we received insight into the role of CB_1 receptor activation in diabetic retinopathy. Deletion of the CB_1 receptor or treatment with a CB_1 receptor antagonist prevented retinal cell death in a murine diabetes model.[85] Treatment of diabetic mice or human retinal cells with CB_1 receptor antagonists after exposure to high glucose levels attenuated oxidative/nitrative stress, reduced NF-κB activation and adhesion molecule levels, and attenuated MAPK activation. These observations were supported by the fact that hyperglycemia up-regulated CB_1 receptor expression and induced apoptosis in retina pigment epithelial cells, effects that were preventable with a CB_1 receptor antagonist.[86] Interestingly, hyperglycemia also decreased FAAH expression, leading to a locally increased concentration of AEA and thereby increasing apoptosis via CB_1 receptor signaling.

The effect of CBD was also examined in experimental diabetic retinopathy. CBD was able to reduce oxidative stress, inflammation, cell death, and vascular hyperpermeability associated with diabetes.[12] Consistent with these findings, CBD also inhibited p38-MAPK signaling. Furthermore, CBD also attenuated high glucose–induced endothelial cell dysfunction, ROS generation, and barrier disruption in primary human coronary artery endothelial cells.[52] The protective effects of CBD on retinal cell death were, at least in part, due to the reduction of tyrosine nitration of glutamine synthase in macroglial cells, thereby preventing the accumulation and excitotoxicity of glutamine through *N*-methyl-D-aspartate receptors.[87]

Diabetic Neuropathy

Approximately 60% to 70% of people with diabetes have some kind of nervous system damage.[18] The typical presentation is chronic, length-dependent sensorimotor neuropathy, which develops in a background of long-standing hyperglycemia and is associated with alterations of microvessels; it can be stabilized with rigorous glycemic control.[88] Autonomic dysfunction and pain may develop over time as well.[88,89]

CB_1 receptors are widely expressed throughout the central and peripheral nervous systems, whereas CB_2 receptors are primarily restricted to the cells of the peripheral nervous system, microglia, and dorsal horn neurons. ECs are retrograde messengers with agonistic activity on presynaptic CB_1 receptors, slowing neurotransmission. A good example of this effect is the suppression of nociceptive transmission in the periphery at the level of the posterior horn of the spinal cord.[90] It has been proven that these peripheral CB_1 receptors play a key role in cannabinoid-induced analgesia.[91] Interestingly, although CB_1 and CB_2 agonists are effective in animal models of acute and chronic pain, in clinical trials, they only perform well in patients with chronic pain syndrome.[92] Sativex spray containing THC and CBD is already approved for the treatment of pain in patients with multiple sclerosis

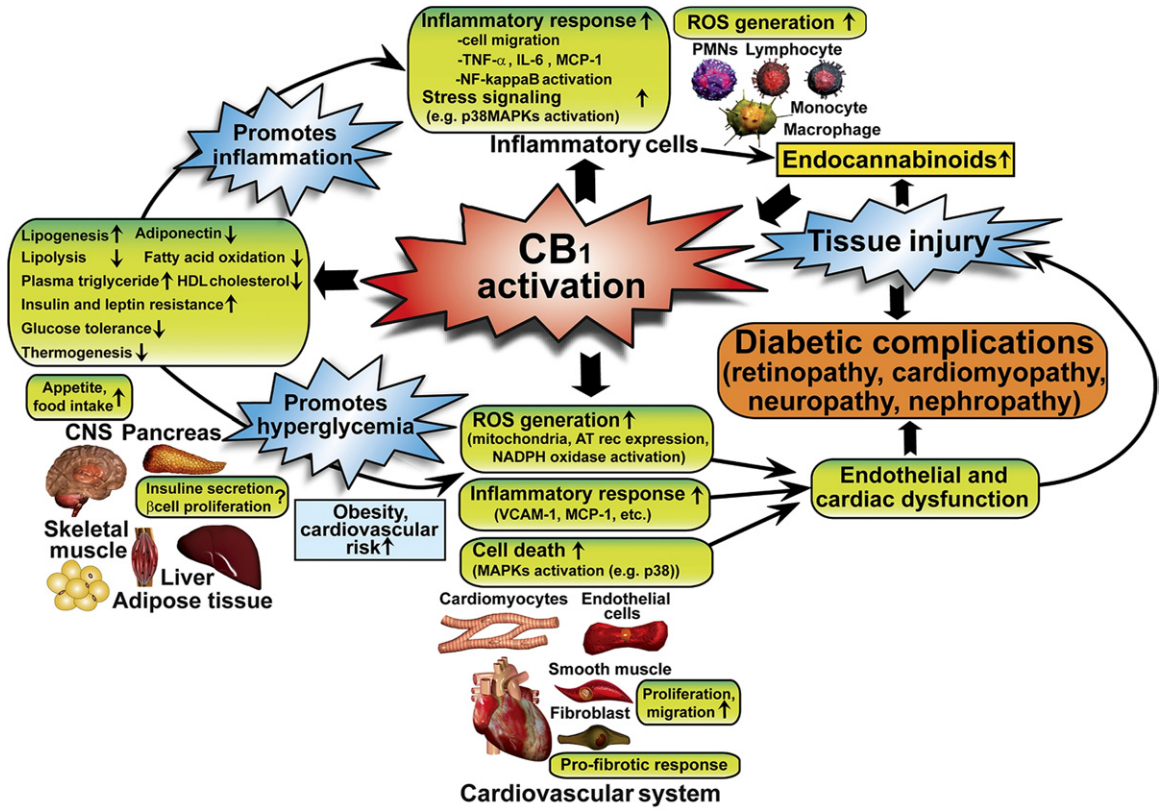

Figure 1. Effects of CB_1 receptor activation on diabetes and diabetic complications. CB_1 receptor activation may indirectly (via its metabolic consequences) or directly enhance diabetes-associated inflammation and ROS generation, promoting tissue injury and the development of diabetic complications. AT rec, angiotensin II receptor type 1; CNS, central nervous system; PMNs, polymorphonuclear leukocytes.

and cancer pain unresponsive to opioid therapy in Canada, the United Kingdom, and Spain.

The first indication of the role of the ECS in diabetic neuropathy came from a murine diabetes model. A dual CB_1/CB_2 receptor agonist inhibited capsaicin-induced calcitonin gene–related peptide release, a measure of sensory neuron function, which was prevented by a CB_1 antagonist.[93] AEA also inhibited capsaicin-induced calcitonin gene–related peptide release in a non-CB_1/CB_2 receptor–dependent fashion, which was interestingly lacking in diabetic mice. Mechanical allodynia in diabetic rats can also be attenuated by treatment with a nonselective cannabinoid agonist.[94] A highly significant finding was that both CB_1 and CB_2 agonists demonstrated antinociceptive effects in mice with streptozotocin-induced diabetes, and there were no pronociceptive effects for either CB_1 or CB_2 antagonists.[95] Even more promising is (in terms of developing and using CB_1 antagonists in the treatment of primary diabetes and diabetic complications) that subchronic CB_1 receptor antagonism has been shown to evoke a κ-opiate–dependent analgesia by increasing the transcription of genes encoding the opioid system in the spinal cord.[96]

Both *in vitro* and *in vivo* findings regarding the role of cannabinoid receptors in the pathogenesis of diabetic peripheral neuropathy are contradictory. CB_1 receptor expression has been shown to be down-regulated in PC-12 cells exposed to high glucose levels and in dorsal root ganglia removed from diabetic rats[97]; the synthetic cannabinoid HU-210 was able to restore impaired nerve growth factor–induced neurite outgrowth in cells exposed to high glucose levels in a CB_1 receptor–dependent manner,[98] consistent with the earlier finding that HU-210 attenuates neural damage.[99] *In vivo*, however, the CB_1 receptor antagonist RIO shows a beneficial effect in diabetic peripheral neuropathy.[100,101] RIO improves de-

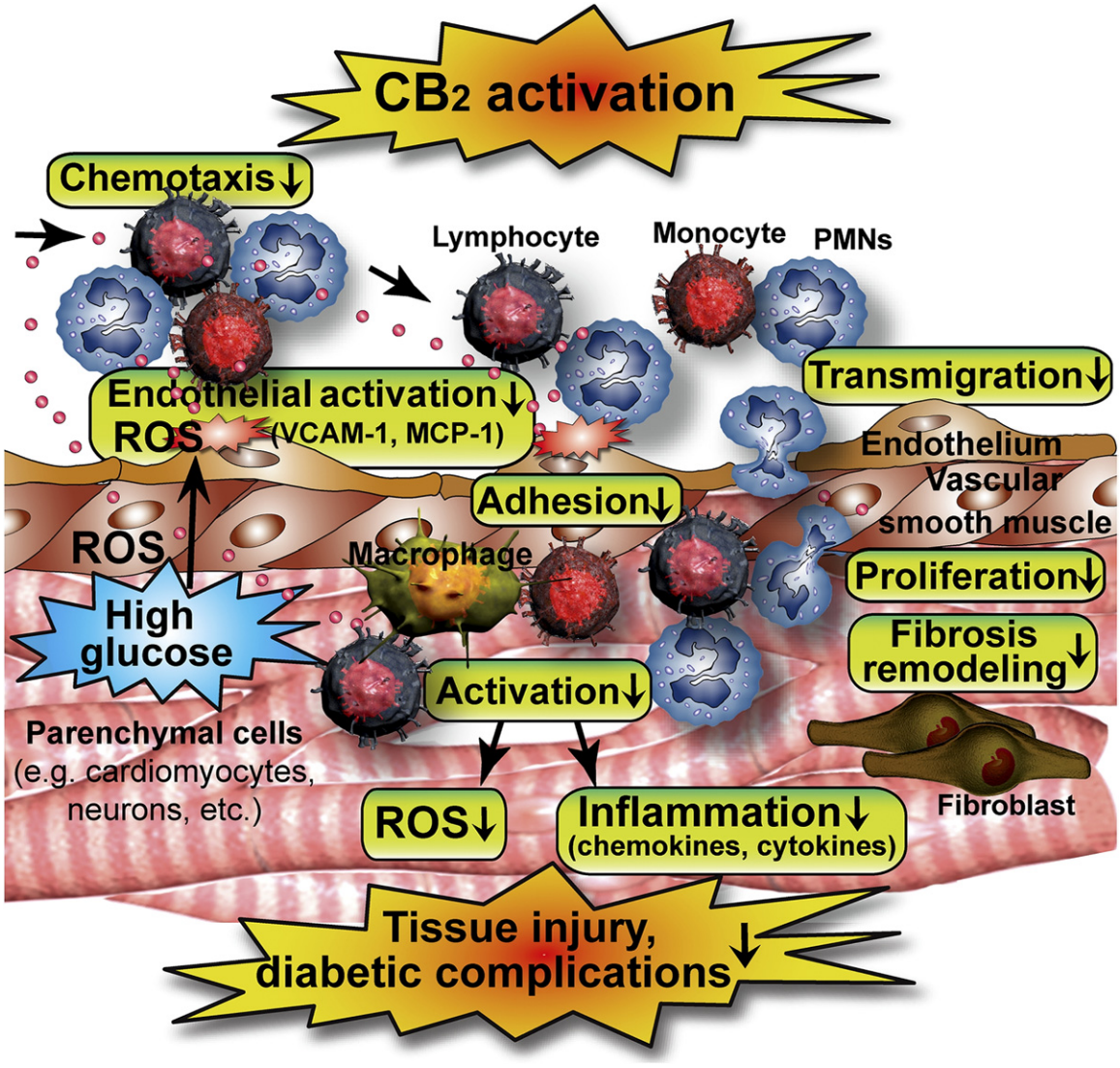

Figure 2. Possible beneficial effects of CB_2 receptor activation on diabetes and diabetic complications. CB_2 receptor stimulation may exert beneficial effects against various diabetic complications by attenuating high glucose–induced endothelial cell activation and inflammatory response; chemotaxis, transmigration, adhesion, and activation of inflammatory cells; and subsequent proinflammatory responses and ROS generation. PMNs, polymorphonuclear leukocytes; VCAM-1, vascular adhesion molecule-1.

creased intraepidermal nerve fiber density and alleviates increased current perception threshold, which is closely associated with the attenuation of skin capillary loss, increase in blood flow, and reduction of TNF-α levels.[101] RIO also ameliorates mechanical allodynia in diabetic mice, reduces oxidative stress in peripheral nerves, inhibits TNF-α overproduction in the spinal cord, and restores NGF content.[100] The alleviation of mechanical allodynia with RIO was attributed to diminished sensitization of the transient receptor potential vanilloid receptor via CB_1 receptor antagonism.[102]

In summary, CB_1 receptor antagonism appears to be a viable option for halting the progression of diabetic neuropathy and may provide some analgesic effects through a κ-opiate–dependent pathway. The natural cannabinoid CBD offers a further possible therapeutic advantage because it was able to attenuate the development of neuropathic pain. This effect was associated with the restriction in the elevations of microglial density in the spinal cord and of phosphorylated p38-MAPK.[95] The first clinical trial with Sativex has already been conducted in patients with painful diabetic neuropathy.[103] Although the trial failed to show any advantage compared with placebo treatment, further analysis is needed because several confounding factors were present.

Conclusion and Perspectives

Although there is much controversy in the field of EC research, experimental evidence and clinical trials have clearly shown that ECS plays a key role in the development of primary diabetes and various diabetic complications. Although inhibition of CB_1 receptors has proven to be effective in clinical trials of obesity and metabolic syndrome, this approach has ultimately failed because of increasing patient anxiety. However, recent preclinical studies clearly showed that peripherally restricted CB_1 antagonists may represent a viable therapeutic strategy to avoid the previously mentioned adverse effects.[25,30] Importantly, CB_1 inhibition, as discussed in this review, may also directly attenuate inflammatory responses and ROS and reactive nitrogen species generation in endothelial, immune, and other cell types, as well as in target tissues of diabetic complications, far beyond its known beneficial metabolic consequences. The main effects of CB_1 receptor activation on the development of diabetes and diabetic complications are summarized in Figure 1. CB_2 agonists may exert beneficial effects on diabetes and diabetic complications by attenuating inflammatory response and ensuing oxidative stress (Figure 2). Natural cannabinoids, such as CBD and THCV, also have tremendous therapeutic potential. CBD is a potent antioxidant and anti-inflammatory agent that does not appear to exert its beneficial effects through conventional CB receptors[104] and is already approved for human use. THCV and its derivatives, which may combine the beneficial effects of simultaneous CB_1 inhibition and CB_2 stimulation, are still under intense preclinical investigation. It will be interesting to see how newly developed, peripherally restricted CB_1 receptor antagonists and/or CB_2 receptor agonists and certain natural cannabinoids, such as CBD and THCV, will influence the clinical outcomes of diabetic patients. We hope that some of these new approaches will be useful in clinical practice in the near future to aid patients with diabetes.

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