Endocannabinoids and Liver Disease.

**I. Endocannabinoids and their receptors in the liver**

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**Mallat A, Lotersztajn S.** Endocannabinoids and Liver Disease. I. Endocannabinoids and their receptors in the liver. *Am J Physiol Gastrointest Liver Physiol* 294: G9–G12, 2008. First published November 1, 2007; doi:10.1152/ajpgi.00467.2007.—Cannabinoid receptors (CB1 and CB2) and their endogenous ligands (endocannabinoids) have recently emerged as novel mediators of liver diseases. Endogenous activation of CB1 receptors promotes nonalcoholic fatty liver disease (NAFLD) and progression of liver fibrosis associated with chronic liver injury; in addition, CB1 receptors contribute to the pathogenesis of portal hypertension and cirrhotic cardiomyopathy. CB2 receptor-dependent effects are also increasingly characterized, including antifibrogenic effects and regulation of liver inflammation during ischemia-reperfusion and NAFLD. It is likely that the next few years will allow us to delineate whether molecules targeting CB1 and CB2 receptors are useful therapeutic agents for the treatment of chronic liver diseases.

**Cannabinoid receptors**

**THE MEDICINAL PROPERTIES of cannabis (Cannabis sativa, marijuana) have been known for millennia, as shown by reports from China and India underscoring its analgesic, antietiemic, and appetite-stimulating properties. During the 19th century, the prescription of cannabis gained popularity for a variety of conditions ranging from epilepsy to rheumatism and abdominal symptoms. Concerns about abuse led to discontinuation of therapeutic use in the 1940s. The characterization of marijuana-derived bioactive molecules began during the early 20th century with the identification of several hydrophobic compounds and culminated in 1964 with the isolation of Δ9-tetrahydrocannabinol (THC), the main psychoactive constituent of the plant. Subsequent studies identified over 60 other phytocannabinoids and allowed the synthesis of active analogs with varying potencies. This step was critical in the identification of the endocannabinoid system, comprising specific cannabinoid binding sites (CB1 and CB2), their endogenous ligands (endocannabinoids), and synthetic and degradative pathways (for reviews, see Refs. 14, 15, 21).

**The Endocannabinoid System**

**Cannabinoid receptors.** Specific binding sites for THC were initially identified in the brain and led to the cloning of a CB1 receptor from a rat brain library (17). Thereafter, a second receptor was cloned from a human promyelocytic cDNA library and designated CB2, based on its 44% homology with CB1 and similar ligand profile (19). Both receptors belong to the family of G protein-coupled receptors and show a wide distribution, with distinct specificities. CB1 is the most abundant G protein-coupled receptor in the brain and exclusively accounts for the psychoactive effects of cannabinoids. Aside from its abundance in the central nervous system, the CB1 receptor is widely distributed at significant levels, particularly in organs that control energy balance. CB2 receptors are expressed at lower levels than CB1 receptors and predominate in cells of the immune system (13). In addition, CB2 receptors are being increasingly detected in a variety of peripheral tissues, particularly in pathological conditions. Intracellular CB1- and CB2-dependent signaling pathways include Gi/o-dependent inhibition of adenyl cyclase, stimulation of MAP kinase, PI3-kinase and FAK pathways, and CB1-specific modulation of calcium and potassium channels. G protein-independent pathways have also been identified, including activation of sphingomyelinase, and induction of nitric oxide synthase or cyclooxygenase type 2. Beyond CB1 and CB2 receptors, pharmacological evidence supports the existence of additional cannabinoid receptors, and along these lines, GRP55 was recently described as a novel cannabinoid receptor (23). Moreover, endocannabinoids may also bind vanilloid receptors and peroxisome-proliferator activated receptors. Finally, cannabinoids also display receptor-independent properties linked to their high lipophilicity (for reviews, see Refs. 14, 15, 21).

**Endocannabinoids.** Characterization of specific receptors for plant-derived cannabinoids was followed in the 1990s by the isolation of two endogenous arachidonic acid-derived ligands, anandamide (arachidonoyl ethanolamide) and 2-arachidonoyl glycerol (2-AG) (14, 15, 21). Other endocannabinoids (i.e., noladin ether, virodhamine, N-arachidonoyl dopamine) were subsequently identified, but their functions and mechanisms of action remain poorly characterized. Anandamide and 2-AG are synthetized on demand from distinct precursors, via phospholipid-dependent pathways involving phospholipase D for anandamide and diacylglycerol lipase for 2-AG. Clearance of anandamide relies on its cellular uptake by a specific transporter or by additional pathways, prior to catabolism by fatty acid amide hydrolase (6, 11). Hydrolysis of 2-AG follows a distinct pathway involving monoacylglycerol lipase (14, 15, 21). Anandamide displays a higher affinity for CB1 than CB2 receptors and is therefore considered a major endogenous CB1 ligand. 2-AG shows a distinct ligand binding profile, characterized by a similar affinity for CB1 and CB2 receptors (13).

The endocannabinoid system as a ubiquitous mediator of biological pathways in health and diseases. An understanding of the crucial role of cannabionoids in health and diseases has...
been rapidly gained from novel tools permitting the identification and functional characterization of cannabinoid receptors. Consequently, several studies have shown that, in addition to their anticipated CB1-dependent central effects, cannabinoids also display a wide variety of CB1- or CB2-mediated peripheral functions, including regulation of energy balance, immune and inflammatory responses and bone mass, as well as antitumor properties and vasoregulatory and lipogenic effects (21). These findings have opened several novel therapeutic strategies, as illustrated last year by the European Agency for the Evaluation of Medicinal Products approval of the CB1 antagonist rimonabant for the treatment of overweight/obesity and associated comorbidities (for a review, see Ref. 16), as well as its ongoing evaluation for the management of smoking or alcohol dependence. In addition, several other compounds with CB1 antagonistic properties are in clinical development. Therapeutic applications of CB2 agonists are also the source of wide interest, particularly in view of their lack of psychoactive effects. Hence, beneficial effects might be expected in atherosclerosis, in osteoporosis, or as analgesics. However, selective CB2 molecules have not reached the clinical stage of development, as yet.

The Endocannabinoid System in the Pathophysiology of Liver Diseases

Accumulating evidence indicates that the cannabinoid system plays a crucial role in the pathophysiology of liver diseases, both as a key player in hepatic injury and as a mediator of complications of cirrhosis. These findings came out somewhat surprisingly, given the low physiological level of expression of CB1 and CB2 receptors in the adult liver. Indeed, whereas the embryonic mouse liver transiently expresses CB2 receptor mRNA, adult liver displays a faint expression of CB1 receptors in endothelial cells and hepatocytes. Nonetheless, the normal liver produces endocannabinoids (14, 15), probably originating from both hepatocytes and nonparenchymal cells (2).

Alcohol abuse, viral hepatitis, and nonalcoholic fatty liver are the predominant causes of chronic liver diseases. Schematically, these conditions generate hepatocyte injury and inflammation, thereby activating liver fibrogenesis. Progression of fibrosis disrupts the liver architecture, ultimately leading to cirrhosis and the life-threatening complications of liver failure and portal hypertension, as well as to incident hepatocellular carcinoma (12). Strikingly, recent studies indicate that the endocannabinoid system is highly upregulated during chronic liver diseases and affects multiple steps of this sequence, including hepatocyte injury and inflammation, fibrogenesis, as well as portal hypertension, cirrhotic cardiomyopathy, and hepatic encephalopathy.

Endocannabinoid effects in immune cells: implication for inflammatory liver diseases. CB2 receptors are known modulators of immune and inflammatory responses in several tissues (10). In an anticipated Themes article in this series, Drs. P. Pacher and B. Gao plan to review data demonstrating the role of CB2 receptors in the interplay between various inflammatory cells and endothelium, as exemplified in experimental hepatic ischemia-reperfusion (I/R) injury. I/R injury is a multifactorial process triggered when the liver is transiently subjected to a reduction of blood supply followed by reperfusion, as occurs during liver surgery or liver transplantation. Liver injury is associated with Kupffer cell activation, the release of proinflammatory cytokines, and generation of reactive oxygen species. These events subsequently promote infiltration of the liver by activated polymorphonuclear and neutrophil-dependent liver dysfunction.

In their studies, Batkai et al. (2) showed that I/R is associated with a dramatic induction of hepatic expression of anandamide and 2-AG, correlated to the extent of liver damage. In addition, activation of CB2 receptors protects from hepatic I/R damage by attenuating liver injury, hepatic inflammation, and oxidative stress. These data therefore point out to the central role of CB2 receptors in the regulation of inflammation, as previously documented in other tissues. Further studies should delineate the role of CB2 receptors in the inflammatory response associated with chronic liver diseases, such as viral hepatitis and alcoholic or nonalcoholic liver disease.

The endocannabinoid system and nonalcoholic fatty liver disease. Nonalcoholic fatty liver disease (NAFLD) is closely linked to the metabolic syndrome and currently accounts for a rising cause of liver injury, with a 20–30% prevalence in Western countries. The spectrum of the disease ranges from simple steatosis, a condition generally associated with a benign liver outcome, to steatohepatitis, an entity that comprises steatosis, liver inflammation and activation of fibrogenic pathways and carries a 20% risk of cirrhosis after 10–20 years. Later in this Themes series, Dr. G. Kunos plan to review evidence indicating that the endocannabinoid system plays a central role in the pathogenesis of NAFLD. The pioneering work of Osei Hyiaman and colleagues showed that activation of CB1 receptors enhances experimental steatosis via CB1-dependent central orexigenic properties as well as peripheral lipogenic effects in hepatocytes (20). In keeping with these data, a recent study in obese fa/1a rats demonstrated that rimonabant prevents the development of steatosis and reduces features of the metabolic syndrome (4). Clinical evidence also strongly supports the role of the endocannabinoid system in nonalcoholic fatty liver disease. Indeed, pivotal trials of rimonabant in obese/obese patients showed that the drug significantly decreases body weight and reduces the prevalence of the metabolic syndrome (16), suggesting that the drug may reduce metabolic steatosis. However, this issue was not addressed in the corresponding studies. We therefore took advantage of the high prevalence of steatosis in patients with chronic hepatitis C and investigated the impact of cannabis use on the severity of steatosis in these patients. By logistic regression analysis, daily cannabis use was identified as a predictor of severe steatosis, together with other known independent factors of steatosis in hepatitis C (8). Altogether, these findings indicate that CB1 receptors mediate metabolic steatogenesis in the liver, by central and peripheral hepatic effects. However, aside from lipogenic effects in the liver, CB1 receptors might also affect lipolysis in the adipose tissue, thereby enhancing the influx of triglycerides in the liver. Therefore, additional studies are needed to precisely define mechanisms underlying CB1-dependent steatogenic effect.

Another important issue is whether CB2 receptors may also participate to the pathogenesis of fatty liver disease, given their well-known effects on the inflammatory response in other organs (10). A recent study reported increased hepatic CB2 receptor expression in patients with NAFLD (18), suggesting...
that these receptors may contribute to disease progression. We therefore evaluated the impact of CB2 antagonism on obesity-induced NAFLD, and found that genetic inactivation of CB2 receptors reduces obesity and insulin resistance. Moreover, CB2 antagonism also blunts hepatic steatosis, following inhibition of obesity-associated inflammation in the adipose tissue (3). These results unravel an unexpected role of CB2 receptors in the control of disorders associated with the metabolic syndrome. They also provide additional evidence supporting the potential involvement of CB2 receptors in inflammatory liver diseases.

Endocannabinoids in the pathogenesis and treatment of liver fibrosis. Liver fibrosis is an established precursor of poor outcome in chronic liver diseases. Accordingly, mechanisms governing liver fibrogenesis have been extensively studied over the past 20 years (12). In an upcoming review in this Themes series, Drs. S. Siegmund and R. Schwabe plan to summarize the key concepts of liver fibrogenesis and recent studies demonstrating the strong impact of the endocannabinoid system in this setting, via cannabinoid receptor-dependent (7, 9, 25) and independent mechanisms (24).

We recently showed that CB1 and CB2 receptors are markedly upregulated in cirrhotic human surgical liver samples, a finding consistent with the marked elevation of circulating levels of anandamide and of hepatic 2-AG described in cirrhotic patients and experimental models of liver fibrosis, respectively (1, 24). These results strongly suggest a role for the endocannabinoid system in liver fibrogenesis. We subsequently demonstrated that CB2 receptors signal antifibrogenic responses, on the basis of experiments in which CB2 knockout mice exposed to carbon tetrachloride showed enhanced liver fibrosis and increased liver fibrogenic cell accumulation compared with wild-type animals. In vitro experiments confirmed these data and further demonstrated that CB2 receptor activation induces growth inhibition and apoptosis of cultured liver fibrogenic cells, following activation of cyclooxygenase-2 and oxidative stress, respectively (9). By using similar approaches, CB1 receptors were found to signal profibrogenic responses. Administration of the CB1 antagonist rimonabant or genetic inactivation of CB1 receptors inhibited fibrosis progression in three models of chronic liver injury, by a mechanism involving reduced proliferation and increased apoptosis of liver fibrogenic cells (25). In aggregate, these results indicated that CB1 and CB2 receptors exert opposite effects on experimental liver fibrosis and therefore raised the issue of the net impact of endocannabinoid signaling on liver fibrogenesis in a clinical setting. The question was addressed in an epidemiological study designed to evaluate the impact of cannabis use on fibrosis severity in 270 patients with chronic hepatitis C. Analysis indicated that daily cannabis use over the span of the disease is an independent predictor of fibrosis severity (7). Altogether, these studies indicate that the endocannabinoid tone is exacerbated during liver fibrogenesis and invite to evaluate the efficiency of available CB1 antagonists as antifibrogenic molecules.

Endocannabinoids as mediators of vascular and cardiac abnormalities in cirrhosis. Several studies have shown that portal hypertension associated with cirrhosis is partly due to a deregulation of pathways controlling vascular reactivity, leading to mesenteric vasodilation. In decompensated cirrhosis, the hyperdynamic circulation resulting from peripheral vasodilation may be associated with latent heart dysfunction, known as cirrhotic cardiomyopathy.

Several lines of evidence, which Drs. S. Gaskari and S. Lee plan to review in this Themes series, indicate that the endocannabinoid system contributes to the hemodynamic alterations associated with cirrhosis. Indeed, endocannabinoids trigger vasorelaxing effects, and several studies have demonstrated that an upregulated CB1-dependent cannabinoid tone contributes to the pathogenesis of portal hypertension, via enhanced mesenteric vasodilation (1, 22). Recent reports additionally documented an increased expression of the cardiac endocannabinoid system in experimental models of cirrhosis, associated with a CB1-dependent impairment of cardiac contractility (5).

Future Directions

As outlined above and discussed in details in the forthcoming issues, growing evidence indicates that endocannabinoids affect several physiopathological processes associated with acute or chronic liver disease. CB1 receptors are crucial mediators of nonalcoholic fatty liver disease and play a key role in liver fibrogenesis associated with chronic liver injury. Moreover, CB1 antagonism has proved useful in the management of experimental portal hypertension and cirrhotic cardiomyopathy. These data indicate that endogenous activation of CB1 receptors triggers several deleterious effects, enhancing progression of chronic liver disease to cirrhosis and its complications. Recent evidence also indicates that CB2 receptors play a key role in regulation of the liver inflammatory response. Potential therapeutic indications will undoubtedly require additional preclinical studies to precisely delineate the conditions associated with pro- or anti-inflammatory effects of CB2 activation. Additional hepatic functions of cannabinoids may also soon arise, since recent studies have reported an upregulation of CB1 and CB2 receptors in hepatocellular carcinoma (26). It is likely that the next few years will allow us to delineate whether molecules targeting this system are useful therapeutic agents for the treatment of chronic liver diseases.

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