Published in final edited form as:
Dig Liver Dis. 2011 March ; 43(3): 188–193. doi:10.1016/j.dld.2010.08.010.

Recent advances in the understanding of the role of the endocannabinoid system in liver diseases

Li Huang[1,2,3], **Matthew A. Quinn**[1,2], **Gabriel A. Frampton**[1], **L. Eric. Golden**[1], and **Sharon DeMorrow**[1,2]

[1] Department of Internal Medicine, Texas A&M Health Science Center College of Medicine, Temple Texas

[2] Digestive Disease Research Center, Scott & White Hospital, Temple Texas

[3] Department of Hepatobiliary Surgery, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Abstract

Endocannabinoids are ubiquitous signaling molecules that exert their effects through a number of specific cannabinoid receptors. Recent studies have indicated that this endocannabinoid system is involved in the pathophysiological processes associated with both acute and chronic liver diseases as well as in the complications that arise from these diseases such as hepatic encephalopathy and cardiac problems. Targeting this signaling system has been useful in ameliorating some of the symptoms and consequences in experimental models of these liver diseases. This review summarizes the recent advances into our knowledge and understanding of endocannabinoids in liver diseases and highlights potential novel therapeutic strategies that may prove useful to treat these diseases

Introduction

Marijuana and its derivatives have been used in medicine for centuries; however, it was not until the isolation of the psychoactive component of Cannabis sativa ($\Delta;^{9}$-tetrahydrocannabinol; THC) and the subsequent discovery of the endogenous cannabinoid signaling system that research into the therapeutic value of cannabinoids re-emerged. Ongoing research is determining that regulation of the endocannabinoid system may be effective in the treatment of pain [1,2], glaucoma [3], and neurodegenerative disorders such as Parkinson's disease [4] and multiple sclerosis [5]. In addition, cannabinoids might be effective antitumoral agents because of their ability to inhibit the growth of various types of cancer cell lines in culture [6–9] and in laboratory animals [10].

The endogenous cannabinoid system consists of the cannabinoid receptors, their endogenous ligands (endocannabinoids) and the proteins for their synthesis and inactivation [11]. The cannabinoid receptors are seven-transmembrane-domain proteins coupled to $G_{i/o}$ type G-

Corresponding Author: Sharon DeMorrow, Ph.D., Department of Internal Medicine, Scott and White Hospital and Texas A&M Health Science Center, College of Medicine. Medical Research Building 702 SW H.K. Dodgen Loop, Temple, TX, 76504, Phone: 254-724-6240, Fax: 254-724-8070, demorrow@medicine.tamhsc.edu.

The authors of this manuscript have no conflicts of interest or commercial relationships to disclose.

proteins [11]. To date, there are two definitive cannabinoid receptors, Cb1 and Cb2, as well as a putative involvement of the vanilloid receptor VR1. More recently, the orphan receptor GPR55 was shown to function as a novel cannabinoid receptor [12]. Cb1 receptors are found predominantly in the central nervous system, but they can also be found in most peripheral tissues including immune cells, the reproductive system, the gastrointestinal tract and the lungs [13–15]. Cb2 receptors are found predominantly in the immune system; i.e. tonsils, spleen, macrophages and lymphocytes [13–15].

To date, many endocannabinoids, all of which are lipid molecules, have been identified with varying affinities for the receptors. Anandamide (AEA) was the first endogenous ligand to be identified [13], which acts as a partial Cb1 agonist and weak Cb2 agonist. It has also been shown to activate the GPR55 receptor [12]. While the physiological roles of many of the other ligands have not yet been fully clarified, AEA has been implicated in a wide variety of physiological and pathological processes.

Currently, there are two biosynthesis pathways for AEA. The first involving the remodelling of an existing membrane phosphoglyceride. This happens through the calcium-dependent *N*-transacylation of phosphotidylethanolamine with arachidonic acid to form *N*-arachidonyl-phosphatidyl-ethanolamine, which is then hydrolyzed to AEA [11,16]. The enzyme responsible for the catalysis of this pathway is phospholipase D [11]. The second pathway is via the *de novo* synthesis of AEA from arachidonic acid and ethanolamine by the enzyme anandamide amidohydrolase catalyzing the reverse reaction from high levels of ethanolamine [16]. After synthesis, AEA is rapidly inactivated via a tightly controlled series of events involving sequestration by cells and enzymatic hydrolysis. The mechanism of AEA uptake is largely unknown, with some data suggesting that it is via passive diffusion and other data indicating that it is through the presence of an active transporter [17]. Regardless of the mechanism, this uptake is a rapid event with a half-life of approximately 2.5 minutes [16]. After uptake, AEA is hydrolyzed and degraded by the enzyme anandamide amidohydrolase (also called fatty acid amide hydrolase or FAAH) [16].

On the other hand, 2-AG is synthesized from diacylglycerol (DAG) via the actions of sn1-specific DAG lipase in a calcium-dependent fashion [11], although PLC-independent mechanisms for 2-AG formation have also been suggested [11]. In addition, 2-AG can be hydrolyzed either by FAAH or a monoacylglycerol lipase (MGL) enzyme to yield arachidonic acid and glycerol [16].

A summary of the biosynthesis and degradation pathways for both AEA and 2-AG can be found in Figure 1.

Cannabinoid synthesis and degradation in acute and chronic liver diseases

Cannabinoid levels are dysregulated during early stages of various liver diseases in humans [18,19] and in rodent models of liver damage [20,21]. In a recent study, analysis of 18 patients with liver cirrhosis and 14 age-matched healthy controls revealed an increase in plasma concentrations of the endocannabinoid AEA, but not 2-AG, as well as an increase in the endocannabinoid-related molecules oleoylethanolamine and palmitoylethanolamine [18]. This increase correlated with the severity of the liver dysfunction (MELD score) [18] and was paralleled by an increase in AEA content in the liver tissue itself [18]. In addition, a similar increase in hepatic and serum levels of AEA can be seen in acute hepatitis [19]. In mouse models, AEA has been shown to be upregulated in fatty liver [20], whereas the levels of 2-AG are significantly upregulated in acute liver injury induced by bile duct ligation or injection with a single dose of carbon tetrachloride [21].

In some instances, the increase in AEA levels has been shown to be associated with decreased expression of FAAH, rather than the activation of any of the synthesis pathways [20]. Therefore, while no information exists concerning the relative levels of AEA in cholestatic liver diseases, experimental evidence suggests that FAAH mRNA and activity levels are decreased during the early stages of these diseases [22], suggesting that perhaps AEA is upregulated in cholestatic liver diseases.

Endocannabinoids as key regulatory molecules of liver fibrosis

Liver fibrosis is a typical response to chronic liver injury that ultimately leads to further complications such as cirrhosis, liver failure, or liver cancer. The fibrogenic process involves the activation and recruitment of both hepatic stellate cells as well as hepatic myofibroblasts to the injured area, where they synthesize such factors as fibrogenic cytokines, growth factors and inhibitors of matrix degradation [23]. Modulation of the endocannabinoid system has been suggested as a potential strategy for treating liver fibrosis. While both Cb1 and Cb2 expression is upregulated in hepatic myofibroblasts and vascular endothelial cells [24,25], activation of these receptors exert opposing effects on the fibrogenic process. Specifically, Cb1 receptors are expressed in hepatic stellate cells in cirrhotic livers during their transformation into myofibroblasts [25]. Inhibition of Cb1 activity has been shown to have antifibrogenic effects in a number of experimental models of fibrosis [25]. Associated with the antifibrogenic effects of Cb1 inhibition were a reduction in hepatic transforming growth factor-b growth inhibition and increased apoptosis of myofibroblasts [25].

Conversely, Cb2 receptor activation appears to exert antifibrogenic effects [24]. Using Cb2 genetic knockout mice, Julien *et al* [24] demonstrated an augmented fibrogenic response to carbon tetrachloride-induced liver injury [24]. Activation of Cb2 receptors on activated hepatic stellate cells inhibited growth via a COX-2-dependent pathway and increased apoptosis via a mechanism involving increased oxidative stress [24]. Our knowledge of the effects of cannabinoids on liver fibrosis is summarized in Fig 2.

Endocannabinoids mediate hepatic/ischemia reperfusion injury

Ischemia reperfusion injury occurs during myocardial infarction, stroke and organ transplant. The mechanism of injury involves the acute generation of reactive oxygen and nitrogen species that follows the reoxygenation of the tissue, which results in direct tissue injury, cell death and ultimately organ failure [26]. In the liver, ischemia reperfusion injury occurs during liver transplant or during surgery to treat extensive hepatic trauma or resect large intrahepatic lesions [27]. Upon restoring the blood supply, the liver is subjected to a further insult, aggravating the injury already caused by the ischemia [27]. Activation of Cb2 by a synthetic agonist in a mouse model of hepatic ischemia reperfusion injury significantly attenuated the extent of liver damage (assessed by serum levels of liver enzymes) [28]. Associated with this Cb2-mediated protection was a decrease in tumor necrosis factor a in tissue and serum, tissue lipid peroxidation, hepatocyte apoptosis and inflammatory cell infiltration [28].

Endocannabinoids attenuate cholangiocyte proliferation during cholestatic liver diseases

During the course of chronic cholestatic liver diseases such as primary sclerosing cholangitis, primary biliary cirrhosis, liver allograft rejection and graft-versus-host disease, cholangiocytes exhibit marked proliferative capacity followed by cholangiocyte loss [29]. As mentioned above, the expression of FAAH is decreased in cholestatic liver disease,

indicating that AEA is probably increased during the course of these diseases. The role of endocannabinoid signaling in the progression of this process is largely unknown. Using a mouse model of cholestatic liver disease, we have previously shown that chronic AEA treatment *in vivo* inhibited biliary growth after bile duct ligation, which could be inhibited by specific Cb2 antagonists [30]. Coupled to the effects of AEA on cholangiocyte proliferation was an increased accumulation of reactive oxygen species [30]. This, in turn, results in an increase in expression and nuclear translocation of Thioredoxin 1 where it interacts with Ref1 [30]. In addition, increased reactive oxygen species result in an upregulation of c-Fos and c-Jun expression, which together constitute the AP-1 DNA-binding activity. The AEA-induced AP-1 transcriptional activity is inhibited in the absence of the TRX1/Ref1 complex, thus suggesting post-translational control of Thioredoxin 1/Ref1 in AP-1 transcriptional activity. A schematic diagram summarizing these data can be found in Figure 1. These data suggest that therapies designed to modulate the endocannabinoid system may prove beneficial in regulating the cholangiocyte proliferation resulting from biliary obstruction, which can be an early event in cholestatic liver diseases.

The effects of cannabinoids on complications of end-stage liver disease

Hepatic encephalopathy

Hepatic encephalopathy is a complication of both acute and chronic liver failure characterized by neurological symptoms ranging from shortened attention span to stupor and coma [31]. Traditionally, hepatic encephalopathy was thought to involve ammonia neurotoxicity due to its inefficient removal by the damaged liver [32]. However, more recently it has been acknowledged that hepatic encephalopathy involves the dysregulation of many neurotransmitter systems, including the monoaminergic [33–35], opioidergic [36] and GABA-ergic [37,38] systems.

The endocannabinoid 2-AG has been found to be elevated in the brains of mice 3 days after the injection of the hepatotoxin thioacetamide [39]. In addition, treatment with 2-AG ameliorated the neurological symptoms of hepatic encephalopathy in these mice [39], which was more pronounced with co-administration with the Cb1 antagonist SR141716A. This suggests that concerted activation of Cb2 and inhibition of Cb1 receptor function may be an effective treatment for hepatic encephalopathy [39]. In another study, researchers showed an increase in both Cb1 and Cb2 expression in the hippocampus using the same mouse model of hepatic encephalopathy as above [40]. Associated with this increase was an activation of AMP-activated protein kinase [40]. Treatment of these animals with the psychoactive component of marijuana, THC, ameliorated the cognitive and motor function decline seen after thioacetamide treatment [40]. The protective effects of THC were abolished in Cb2-deficient mice [40]. A summary of these findings can be found in Figure 4.

Vascular and cardiac abnormalities in cirrhosis

Cirrhosis and portal hypertension are associated with cardiovascular abnormalities [41]. The pathogenesis of cirrhotic cardiomyopathy is unclear. However, several factors including central neural dysregulation [42] and humoral factors such as nitric oxide likely play a pathogenic role. Recent evidence suggests that cannabinoids may also play a role in the pathogenesis of cardiomyopathy [43].

In bile duct-ligated cirrhotic rats, cardiac responsiveness to b-adrenergic stimulation is blunted [44]. This effect can be reversed by the *in vitro* and *in vivo* administration of a Cb1 antagonist [44,45] and was suggested to be a result of the direct effect on cardiac muscle, rather than an indirect effect of Cb1 inhibition on peripheral vasculature [45]. It is suggested that in the cirrhotic heart, the local overproduction of anandamide exerts a negative inotropic

effect via Cb1 receptors [43]. However, the exact location of the anandamide production in this model is unknown.

Effects of cannabinoids in liver cancer

Hepatocellular carcinoma

Hepatocellular carcinoma is the most predominant liver cancer: it is the fifth most common malignancy in men and the eighth in women worldwide [46]. Despite the treatment options currently available, the prognosis of hepatocellular carcinoma remains poor [47]. The role of cannabinoids in hepatocarcinogenesis is unclear, although Xu *et al* [48] have demonstrated that the expression of cannabinoid receptors Cb1 and Cb2 are increased in cancerous tissue compared to the non-malignant liver tissue [48]. The expression of both Cb1 and Cb2 were closely correlated to histopathological grade, with well-differentiated hepatocellular tumors exhibiting a higher expression and poorly differentiated tumors showing low Cb1 immunoreactivity [48]. Furthermore, there was no correlation between cannabinoid receptor expression and survival rate. However, high Cb1 and Cb2 expression correlated with disease-free survival [48]. Modulation of both cannabinoid receptors by the synthetic cannabinoid WIN 55,212–2 (WIN) induces apoptosis in a hepatocellular carcinoma cell line [49]. Associated with the inhibitory effects of WIN was an up-regulation of death-signaling factors Bax and Bcl-Xs and down regulation of the survival factors Survivin, Hsp72 and Bcl-2 [49]. These effects were via the upregulation of PPARg-mediated transcriptional activity [49]. In parallel, WIN has been shown to sensitize HepG2 cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis [50] via the upregulation of the TRAIL receptor (DR5) [50]. Taken together, these data suggest that cannabinoid receptor expression may be useful as prognostic markers for hepatocellular carcinoma, and that the modulation of cannabinoid receptor activity may be an important therapeutic target for the treatment of hepatocellular carcinoma.

Cholangiocarcinoma

Cholangiocarcinoma arises from the neoplastic transformation of cholangiocytes and can present as intrahepatic, perihilar or distal extrahepatic tumors [51]. Typically, cholangiocarcinomas are adenocarcinomas and have a poor prognosis and limited treatment options. This is due at least in part, to the late presentation of symptoms and the relative resistance to current treatment options [52]. The incidence of both intra-and extra-hepatic cholangiocarcinoma is typically higher in Asian countries [53]. The mortality rates for intrahepatic cholangiocarcinoma have increased since the 1970s, whereas deaths from extrahepatic cholangiocarcinoma have declined in most countries [53]. There is a slight preponderance for cholangiocarcinoma in males [54] and the incidence in both sexes increases with age [53].

We have previously shown the differential effects of anandamide (AEA) and 2-arachidonyl glycerol (2-AG) on cholangiocarcinoma growth *in vitro* [55] using a number of cholangiocarcinoma cell lines and in a xenograft model of cholangiocarcinoma (Figure 5) [55,56]. The growth-promoting effects of 2-AG were found to be via a cannabinoid receptor-independent mechanism involving the disruption of lipid raft structures in the cell membrane [55]. Conversely, the antiproliferative actions of AEA were via a mechanism involving the stabilization of lipid rafts in the plasma membrane and the recruitment of death receptor complexes into these membrane microdomains [55]. Furthermore, we have shown that AEA suppresses tumor growth *in vivo* using a xenograft model of cholangiocarcinoma [56] and that there was a concomitant activation of the non-canonical Wnt pathway via upregulation of Wnt 5a [56]. More recently, we have demonstrated that the antiproliferative actions of AEA are also associated with an increase in Notch 1 expression

and activation, whereas the growth-promoting effects of 2-AG can be associated with an increase in Notch 2 expression and activation [57]. The Notch signal transduction pathways require proteolytic processing of the Notch proteins by a membrane-bound γ-secretase complex [58–61]. The dependence and recruitment of the γ-secretase complex to lipid raft structures has previously been shown to modulate γ-secretase activity [62,63]. Therefore it is conceivable that agents that stabilize or disrupt lipid raft structures such as cannabinoids [55] may indeed also regulate the Notch signaling pathway. The involvement of lipid rafts in the differential activation of the Notch signaling pathways by endocannabinoids is a topic of ongoing research in our laboratory. Furthermore, activation of the Wnt signaling pathway has been shown to overlap and crosstalk with the Notch signaling pathway [64–66]. Indeed, activation of Notch 1 has been shown to upregulate the expression of Wnt5a in a number of cell models [67].

Conclusions

From the work described above it is obvious that there remain large gaps in our knowledge concerning the role of cannabinoids in the pathological processes associated with acute and chronic liver diseases. Modulation of endocannabinoid signaling seems to be a promising target for the treatment of not only the type of liver disease in question (eg cholestatic liver diseases) but may also alleviate the symptoms arising from the complications of acute and chronic liver diseases (eg hepatic encephalopathy). Increased efforts in dissecting the molecular mechanisms by which cannabinoids regulate the pathophysiology of these diseases is necessary to design multifaceted approaches to target key symptoms and consequences of these diseases. In addition, the discovery of the novel endocannabinoid receptor GPR55 brings new and exciting opportunities to target and manipulate the activity of this receptor for the treatment of liver diseases. Research into the role of GPR55 in various liver diseases including liver cancer, is currently underway.

Acknowledgments

This work was supported by an NIH K01 grant award (DK078532), an NIH R03 grant award (DK088012) and a Research Scholar award from the American Cancer Society (RSC 118760) to Dr. DeMorrow, a state scholarship of China Scholarship Council to Dr Huang, and a Scott & White Mentored research award to Mr Golden.

References

1. Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. Nature 1998;394:277–281. [PubMed: 9685157]
2. Manzanares J, Corchero J, Romero J, Fernandez-Ruiz JJ, Ramos JA, Fuentes JA. Pharmacological and biochemical interactions between opioids and cannabinoids. Trends Pharmacol Sci 1999;20:287–294. [PubMed: 10390647]
3. Voth EA, Schwartz RH. Medicinal applications of delta-9-tetrahydrocannabinol and marijuana. Ann Intern Med 1997;126:791–798. [PubMed: 9148653]
4. Piomelli D, Giuffrida A, Calignano A, Rodriguez de Fonseca F. The endocannabinoid system as a target for therapeutic drugs. Trends Pharmacol Sci 2000;21:218–224. [PubMed: 10838609]
5. Baker D, Pryce G, Croxford JL, Brown P, Pertwee RG, Huffman JW, Layward L. Cannabinoids control spasticity and tremor in a multiple sclerosis model. Nature 2000;404:84–87. [PubMed: 10716447]
6. De Petrocellis L, Melck D, Palmisano A, Bisogno T, Laezza C, Bifulco M, Di Marzo V. The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. Proc Natl Acad Sci U S A 1998;95:8375–8380. [PubMed: 9653194]
7. Ruiz L, Miguel A, Diaz-Laviada I. Delta9-tetrahydrocannabinol induces apoptosis in human prostate pc-3 cells via a receptor-independent mechanism. FEBS Lett 1999;458:400–404. [PubMed: 10570948]

8. Sanchez C, de Ceballos ML, del Pulgar TG, Rueda D, Corbacho C, Velasco G, Galve-Roperh I, Huffman JW, Ramon y Cajal S, Guzman M. Inhibition of glioma growth *in vivo* by selective activation of the cb(2) cannabinoid receptor. Cancer Res 2001;61:5784–5789. [PubMed: 11479216]
9. Sanchez C, Galve-Roperh I, Canova C, Brachet P, Guzman M. Delta9-tetrahydrocannabinol induces apoptosis in c6 glioma cells. FEBS Lett 1998;436:6–10. [PubMed: 9771884]
10. Galve-Roperh I, Sanchez C, Cortes ML, del Pulgar TG, Izquierdo M, Guzman M. Anti-tumoral action of cannabinoids: Involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. Nat Med 2000;6:313–319. [PubMed: 10700234]
11. Bisogno T, Ligresti A, Di Marzo V. The endocannabinoid signalling system: Biochemical aspects. Pharmacol Biochem Behav 2005;81:224–238. [PubMed: 15935454]
12. 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]
13. Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 1988;34:605–613. [PubMed: 2848184]
14. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cdna. Nature 1990;346:561–564. [PubMed: 2165569]
15. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–65. [PubMed: 7689702]
16. Di Marzo V, Deutsch DG. Biochemistry of the endogenous ligands of cannabinoid receptors. Neurobiol Dis 1998;5:386–404. [PubMed: 9974173]
17. Glaser ST, Kaczocha M, Deutsch DG. Anandamide transport: A critical review. Life Sci 2005;77:1584–1604. [PubMed: 15979096]
18. Caraceni P, Viola A, Piscitelli F, Giannone F, Berzigotti A, Cescon M, Domenicali M, Petrosino S, Giampalma E, Riili A, Grazi G, Golfieri R, Zoli M, Bernardi M, Di Marzo V. Circulating and hepatic endocannabinoids and endocannabinoid-related molecules in patients with cirrhosis. Liver Int. 2009
19. Biswas KK, Sarker KP, Abeyama K, Kawahara K, Iino S, Otsubo Y, Saigo K, Izumi H, Hashiguchi T, Yamakuchi M, Yamaji K, Endo R, Suzuki K, Imaizumi H, Maruyama I. Membrane cholesterol but not putative receptors mediates anandamide-induced hepatocyte apoptosis. Hepatology 2003;38:1167–1177. [PubMed: 14578855]
20. Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Batkai S, Harvey-White J, Mackie K, Offertaler L, Wang L, Kunos G. Endocannabinoid activation at hepatic cb1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J Clin Invest 2005;115:1298–1305. [PubMed: 15864349]
21. Siegmund SV, Qian T, de Minicis S, Harvey-White J, Kunos G, Vinod KY, Hungund B, Schwabe RF. The endocannabinoid 2-arachidonoyl glycerol induces death of hepatic stellate cells via mitochondrial reactive oxygen species. FASEB J 2007;21:2798–2806. [PubMed: 17440119]
22. Siegmund SV, Seki E, Osawa Y, Uchinami H, Cravatt BF, Schwabe RF. Fatty acid amide hydrolase determines anandamide-induced cell death in the liver. J Biol Chem 2006;281:10431–10438. [PubMed: 16418162]
23. Lotersztajn S, Julien B, Teixeira-Clerc F, Grenard P, Mallat A. Hepatic fibrosis: Molecular mechanisms and drug targets. Annu Rev Pharmacol Toxicol 2005;45:605–628. [PubMed: 15471534]
24. Julien B, Grenard P, Teixeira-Clerc F, Van Nhieu JT, Li L, Karsak M, Zimmer A, Mallat A, Lotersztajn S. Antifibrogenic role of the cannabinoid receptor cb2 in the liver. Gastroenterology 2005;128:742–755. [PubMed: 15765409]
25. Teixeira-Clerc F, Julien B, Grenard P, Tran Van Nhieu J, Deveaux V, Li L, Serriere-Lanneau V, Ledent C, Mallat A, Lotersztajn S. Cb1 cannabinoid receptor antagonism: A new strategy for the treatment of liver fibrosis. Nat Med 2006;12:671–676. [PubMed: 16715087]
26. Teoh NC, Farrell GC. Hepatic ischemia reperfusion injury: Pathogenic mechanisms and basis for hepatoprotection. J Gastroenterol Hepatol 2003;18:891–902. [PubMed: 12859717]

27. Serracino-Inglott F, Habib NA, Mathie RT. Hepatic ischemia-reperfusion injury. Am J Surg 2001;181:160–166. [PubMed: 11425059]
28. Rajesh M, Pan H, Mukhopadhyay P, Batkai S, Osei-Hyiaman D, Hasko G, Liaudet L, Gao B, Pacher P. Cannabinoid-2 receptor agonist hu-308 protects against hepatic ischemia/reperfusion injury by attenuating oxidative stress, inflammatory response, and apoptosis. J Leukoc Biol 2007;82:1382–1389. [PubMed: 17652447]
29. Marzioni M, Saccomanno S, Candelaresi C, Rychlicki C, Agostinelli L, Trozzi L, De Minicis S, Benedetti A. Clinical implications of novel aspects of biliary pathophysiology. Dig Liver Dis 2010;42:238–244. [PubMed: 20167547]
30. DeMorrow S, Francis H, Gaudio E, Ueno Y, Venter J, Onori P, Franchitto A, Vaculin B, Vaculin S, Alpini G. Anandamide inhibits cholangiocyte hyperplastic proliferation via activation of thioredoxin 1/redox factor 1 and ap-1 activation. Am J Physiol Gastrointest Liver Physiol 2008;294:G506–519. [PubMed: 18096608]
31. Magen I, Avraham Y, Berry E, Mechoulam R. Endocannabinoids in liver disease and hepatic encephalopathy. Curr Pharm Des 2008;14:2362–2369. [PubMed: 18781986]
32. Butterworth RF, Giguere JF, Michaud J, Lavoie J, Layrargues GP. Ammonia: Key factor in the pathogenesis of hepatic encephalopathy. Neurochem Pathol 1987;6:1–12. [PubMed: 3306479]
33. Mousseau DD, Baker GB, Butterworth RF. Increased density of catalytic sites and expression of brain monoamine oxidase a in humans with hepatic encephalopathy. J Neurochem 1997;68:1200–1208. [PubMed: 9048767]
34. Rao VL, Giguere JF, Layrargues GP, Butterworth RF. Increased activities of maoa and maob in autopsied brain tissue from cirrhotic patients with hepatic encephalopathy. Brain Res 1993;621:349–352. [PubMed: 8242348]
35. Rao VL, Butterworth RF. Alterations of [3h]8-oh-dpat and [3h]ketanserin binding sites in autopsied brain tissue from cirrhotic patients with hepatic encephalopathy. Neurosci Lett 1994;182:69–72. [PubMed: 7891891]
36. Yurdaydin C, Karavelioglu D, Onaran O, Celik T, Yasa MH, Uzunalimoglu O. Opioid receptor ligands in human hepatic encephalopathy. J Hepatol 1998;29:796–801. [PubMed: 9833918]
37. Jalan R, Turjanski N, Taylor-Robinson SD, Koepp MJ, Richardson MP, Wilson JA, Bell JD, Brooks DJ. Increased availability of central benzodiazepine receptors in patients with chronic hepatic encephalopathy and alcohol related cirrhosis. Gut 2000;46:546–552. [PubMed: 10716686]
38. Ahboucha S, Pomier-Layrargues G, Butterworth RF. Increased brain concentrations of endogenous (non-benzodiazepine) gaba-a receptor ligands in human hepatic encephalopathy. Metab Brain Dis 2004;19:241–251. [PubMed: 15554420]
39. Avraham Y, Israeli E, Gabbay E, Okun A, Zolotarev O, Silberman I, Ganzburg V, Dagon Y, Magen I, Vorobia L, Pappo O, Mechoulam R, Ilan Y, Berry EM. Endocannabinoids affect neurological and cognitive function in thioacetamide-induced hepatic encephalopathy in mice. Neurobiol Dis 2006;21:237–245. [PubMed: 16102970]
40. Dagon Y, Avraham Y, Ilan Y, Mechoulam R, Berry EM. Cannabinoids ameliorate cerebral dysfunction following liver failure via amp-activated protein kinase. FASEB J 2007;21:2431–2441. [PubMed: 17431095]
41. Caraceni P, Domenicali M, Giannone F, Bernardi M. The role of the endocannabinoid system in liver diseases. Best Pract Res Clin Endocrinol Metab 2009;23:65–77. [PubMed: 19285261]
42. Song D, Liu H, Sharkey KA, Lee SS. Hyperdynamic circulation in portal-hypertensive rats is dependent on central c-fos gene expression. Hepatology 2002;35:159–166. [PubMed: 11786972]
43. Moezi L, Gaskari SA, Lee SS. Endocannabinoids and liver disease. V. Endocannabinoids as mediators of vascular and cardiac abnormalities in cirrhosis. Am J Physiol Gastrointest Liver Physiol 2008;295:G649–653. [PubMed: 18703639]
44. Gaskari SA, Liu H, Moezi L, Li Y, Baik SK, Lee SS. Role of endocannabinoids in the pathogenesis of cirrhotic cardiomyopathy in bile duct-ligated rats. Br J Pharmacol 2005;146:315–323. [PubMed: 16025138]
45. Moezi L, Gaskari SA, Liu H, Baik SK, Dehpour AR, Lee SS. Anandamide mediates hyperdynamic circulation in cirrhotic rats via cb(1) and vr(1) receptors. Br J Pharmacol 2006;149:898–908. [PubMed: 17043671]

46. Bosch FX, Ribes J, Diaz M, Cleries R. Primary liver cancer: Worldwide incidence and trends. Gastroenterology 2004;127:S5–S16. [PubMed: 15508102]
47. Thomas MB, Zhu AX. Hepatocellular carcinoma: The need for progress. J Clin Oncol 2005;23:2892–2899. [PubMed: 15860847]
48. Xu X, Liu Y, Huang S, Liu G, Xie C, Zhou J, Fan W, Li Q, Wang Q, Zhong D, Miao X. Overexpression of cannabinoid receptors cb1 and cb2 correlates with improved prognosis of patients with hepatocellular carcinoma. Cancer Genet Cytogenet 2006;171:31–38. [PubMed: 17074588]
49. Giuliano M, Pellerito O, Portanova P, Calvaruso G, Santulli A, De Blasio A, Vento R, Tesoriere G. Apoptosis induced in hepg2 cells by the synthetic cannabinoid win: Involvement of the transcription factor ppargamma. Biochimie 2009;91:457–465. [PubMed: 19059457]
50. Pellerito O, Calvaruso G, Portanova P, De Blasio A, Santulli A, Vento R, Tesoriere G, Giuliano M. The synthetic cannabinoid win 55,212–2 sensitizes hepatocellular carcinoma cells to tumor necrosis factor-related apoptosis-inducing ligand (trail)-induced apoptosis by activating p8/ccaat/enhancer binding protein homologous protein (chop)/death receptor 5 (dr5) axis. Mol Pharmacol 2010;77:854–863. [PubMed: 20159939]
51. Alpini G, Prall R, LaRusso NF. The pathobiology of biliary epithelia. The Liver; Biology & Pathobiology 2001;4E:421–435.
52. Sirica AE. Cholangiocarcinoma: Molecular targeting strategies for chemoprevention and therapy. Hepatology 2005;41:5–15. [PubMed: 15690474]
53. Patel T. Worldwide trends in mortality from biliary tract malignancies. BMC Cancer 2002;2:10. [PubMed: 11991810]
54. Tominaga S, Kuroishi T. Biliary tract cancer. Cancer Surv 1994;19–20:125–137.
55. DeMorrow S, Glaser S, Francis H, Venter J, Vaculin B, Vaculin S, Alpini G. Opposing actions of endocannabinoids on cholangiocarcinoma growth: Recruitment of fas and fas ligand to lipid rafts. J Biol Chem 2007;282:13098–13113. [PubMed: 17329257]
56. DeMorrow S, Francis H, Gaudio E, Venter J, Franchitto A, Kopriva S, Onori P, Mancinelli R, Frampton G, Coufal M, Mitchell BM, Vaculin B, Alpini G. The endocannabinoid anandamide inhibits cholangiocarcinoma growth via activation of the non-canonical wnt signaling pathway. Am J Physiol Gastrointest Liver Physiol 2008;295:G1150–1158. [PubMed: 18832445]
57. Frampton G, Coufal M, Li H, Ramirez J, DeMorrow S. Opposing actions of endocannabinoids on cholangiocarcinoma growth is via the differential activation of notch signaling. Experimental Cell Research. 2010 In submission.
58. Kopan R, Goate A. Aph-2/nicastrin: An essential component of gamma-secretase and regulator of notch signaling and presenilin localization. Neuron 2002;33:321–324. [PubMed: 11832221]
59. Lai EC. Notch cleavage: Nicastrin helps presenilin make the final cut. Curr Biol 2002;12:R200–202. [PubMed: 11909545]
60. Lee SF, Shah S, Li H, Yu C, Han W, Yu G. Mammalian aph-1 interacts with presenilin and nicastrin and is required for intramembrane proteolysis of amyloid-beta precursor protein and notch. J Biol Chem 2002;277:45013–45019. [PubMed: 12297508]
61. Li T, Ma G, Cai H, Price DL, Wong PC. Nicastrin is required for assembly of presenilin/gamma-secretase complexes to mediate notch signaling and for processing and trafficking of beta-amyloid precursor protein in mammals. J Neurosci 2003;23:3272–3277. [PubMed: 12716934]
62. Gamerdinger M, Clement AB, Behl C. Effects of sulindac sulfide on the membrane architecture and the activity of gamma-secretase. Neuropharmacology 2008;54:998–1005. [PubMed: 18359496]
63. Ristorcelli E, Beraud E, Mathieu S, Lombardo D, Verine A. Essential role of notch signaling in apoptosis of human pancreatic tumoral cells mediated by exosomal nanoparticles. Int J Cancer 2009;125:1016–1026. [PubMed: 19405120]
64. Collu GM, Brennan K. Cooperation between wnt and notch signalling in human breast cancer. Breast Cancer Res 2007;9:105. [PubMed: 17531087]
65. Katoh M. Transcriptional mechanisms of wnt5a based on nf-kappab, hedgehog, tgfbeta, and notch signaling cascades. Int J Mol Med 2009;23:763–769. [PubMed: 19424602]

66. Nakamura T, Tsuchiya K, Watanabe M. Crosstalk between wnt and notch signaling in intestinal epithelial cell fate decision. J Gastroenterol 2007;42:705–710. [PubMed: 17876539]

67. Koyanagi M, Bushoven P, Iwasaki M, Urbich C, Zeiher AM, Dimmeler S. Notch signaling contributes to the expression of cardiac markers in human circulating progenitor cells. Circ Res 2007;101:1139–1145. [PubMed: 17967789]

68. Izzo AA, Camilleri M. Emerging role of cannabinoids in gastrointestinal and liver diseases: Basic and clinical aspects. Gut 2008;57:1140–1155. [PubMed: 18397936]

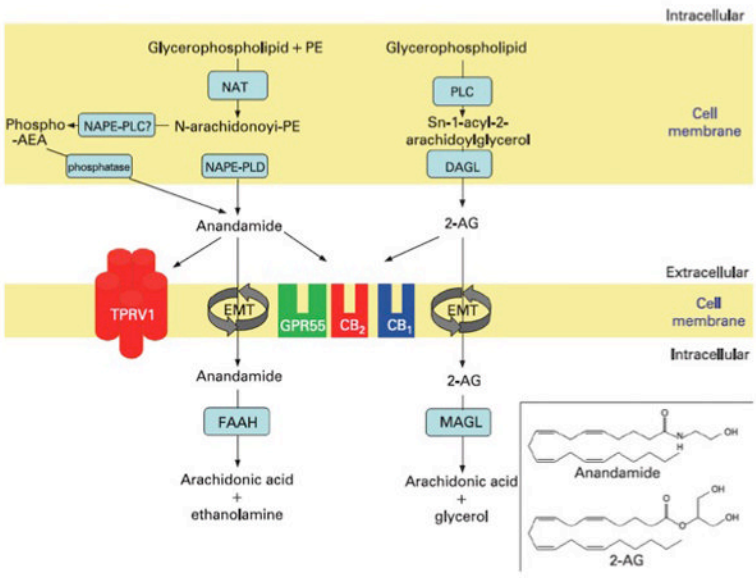

Figure 1.

Biosynthesis and breakdown of the two predominant endocannabinoids, anandamide (AEA) and 2-arachydonoylglycerol (2-AG). The inset shows the chemical structures of AEA and 2-AG. AEA, arachidonoylethanolamine (anandamide); DAGL, diacylglycerol lipase; EMT, endocannabinoid membrane transporter; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAPE, N-acyl-phosphatidylethanolamine; NAPE-PLC, N-acyl-phosphatidylethanolamine-selective phospholipase C; NAPE-PLD, N-acyl-phosphatidylethanolamineselective phospholipase D; NAT, N-acyltransferase; PE, phosphatidylethanolamine; PLC, phospholipase C; TRPV1, transient receptor potential vanilloid type 1. Reproduced from “Emerging role of cannabinoids in gastrointestinal and liver diseases: basic and clinical aspects: AA Izzo and M Camilleri, Gut; 57; 1140–1155, 2008 [68] with permission from BMJ Publishing Group Ltd.

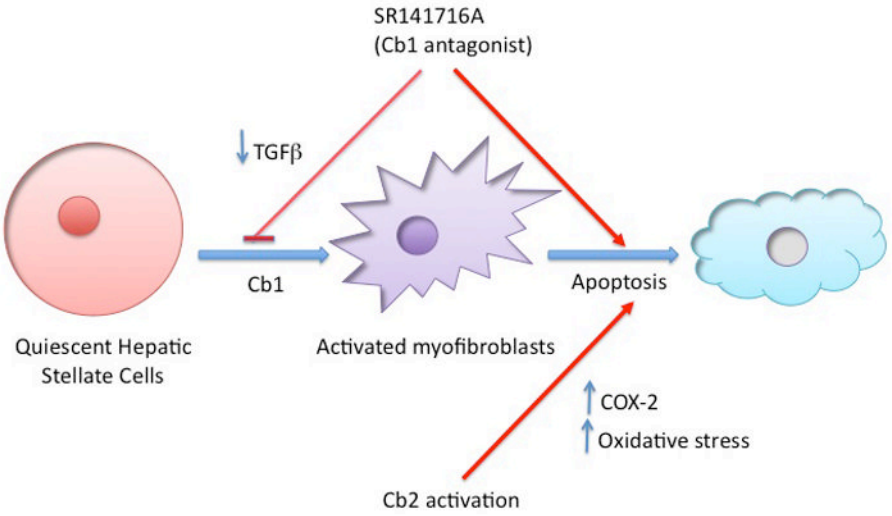

Figure 2.
Schematic diagram depicting the effects of cannabinoids and cannabinoid receptor activation of hepatic stellate cell activation and the resulting liver fibrosis; Cb, cannabinoid receptor; COX-2. Cyclooxygenase 2; TGFb, transforming growth factor b.

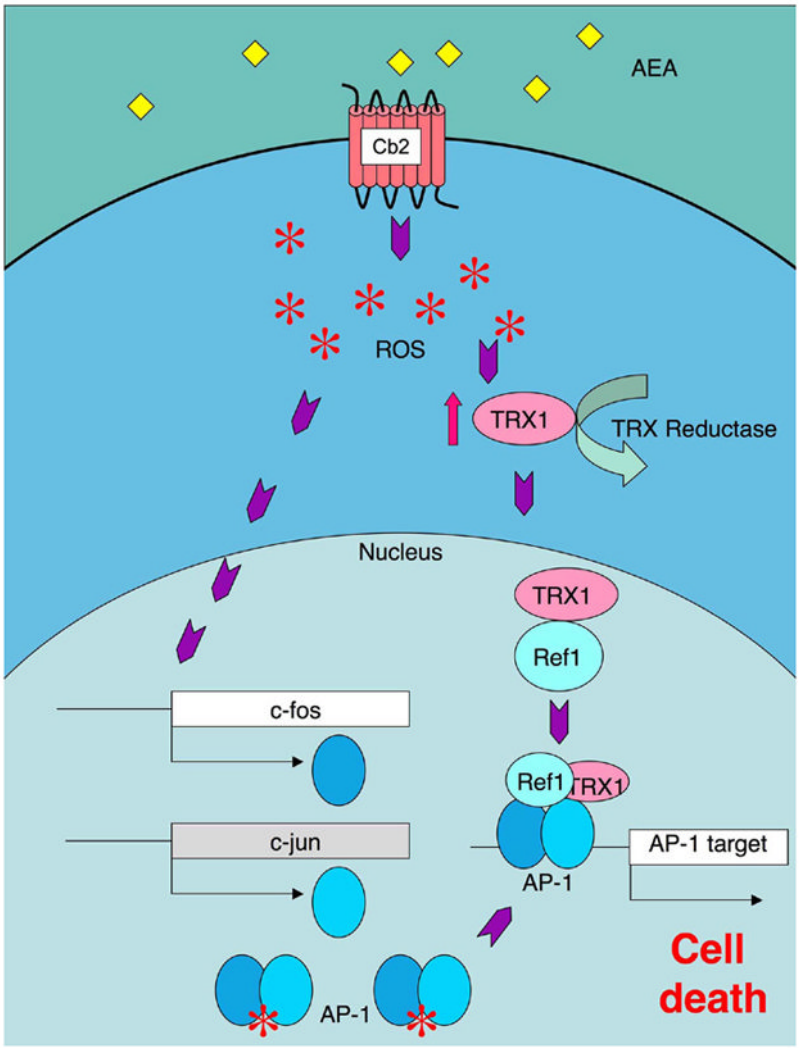

Figure 3.

Schematic diagram of the potential cell signaling mechanisms responsible for the AEA-induced cell death of proliferating cholangiocytes after BDL. Activation of Cb2 by AEA results in increased intracellular ROS accumulation (red asterisks). This, in turn, results in an increased expression and nuclear translocation of TRX1 (but not TRX2) where it interacts with Ref1. In addition, increased ROS results in an upregulation of c-Fos and c-Jun expression, which together constitute the AP-1 DNA-binding activity. However, this transcription factor, under oxidized conditions such as that seen here (i.e., in the presence of increased ROS) fails to retain AP-1 transcriptional activity. This apparent dichotomy is resolved by the reducing properties of the TRX1/Ref1 complex, which restores the AP-1 complex to its reduced form thereby allowing DNA-binding activity and the subsequent transcription of AP-1 target genes that are responsible for the AEA-induced cell death. Reproduced from DeMorrow et al (Am J Physiol Gastrointest Liver Physiol 2008;294:G506–519) [30], with permission from Am Physiol Soc.

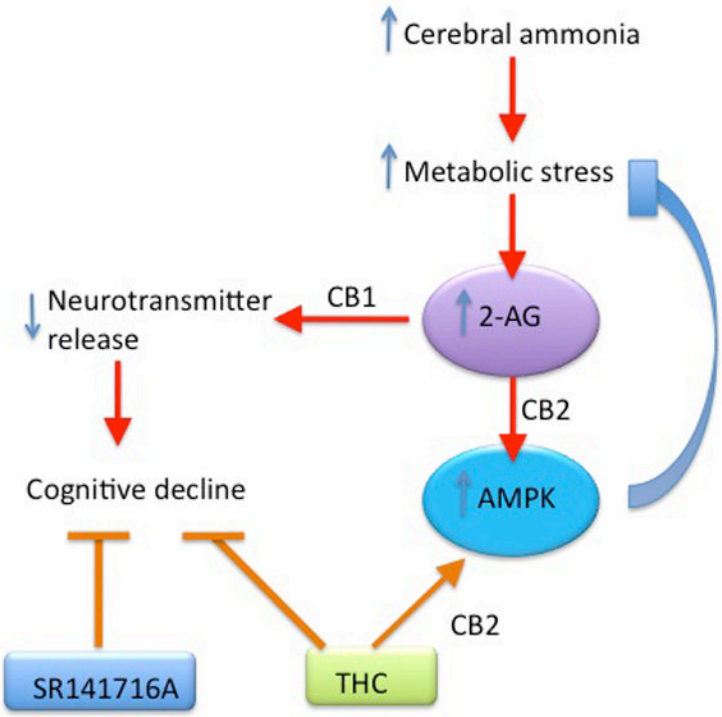

Figure 4.
A schematic representation of current knowledge into the effects of endocannabinoids in hepatic encephalopathy. 2-AG, 2-arachidonyl glycerol; AMPK, AMP activated protein kinase; Cb, Cannabinoid receptor; THC, tetrahydrocannabinol.

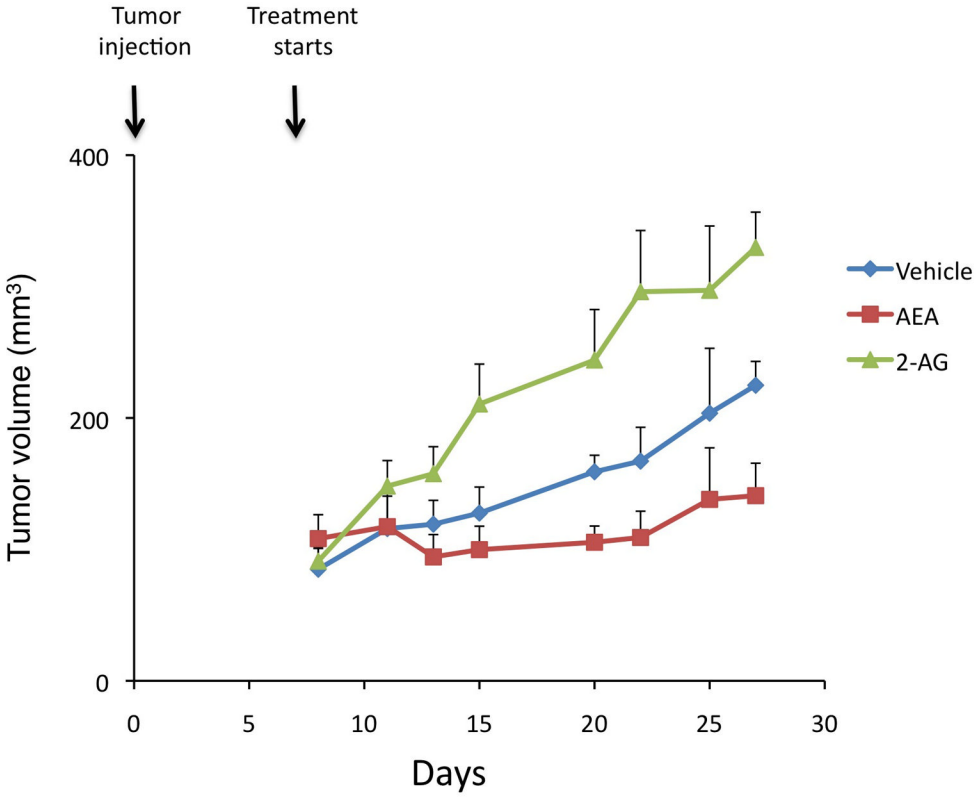

Figure 5.

Opposing effects of the endocannabinoids AEA and 2-AG on cholangiocarcinoma growth occurs in an *in vivo* xenograft model of cholangiocarcinoma. Mz-ChA-1 cells were injected into the flank of athymic mice. After tumors were established, mice were treated with 10 mg/kg/day (ip) AEA, 2-AG or vehicle, three days per week for 28 days and tumor volume assessed.

Reprinted from Frampton et al, [57] Experimental cell research, 2010, 316(9); 1465–1478, with permission from Elsevier.