Endocannabinoids and Liver Disease.

V. Endocannabinoids as mediators of vascular and cardiac abnormalities in cirrhosis

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Submitted 23 May 2008; accepted in final form 6 August 2008

Cirrhosis and portal hypertension are associated with cardiovascular abnormalities. The circulation becomes hyperdynamic, defined as increased cardiac output and decreased peripheral vascular resistance and blood pressure. The peripheral vasodilatation is also found in local vascular beds such as the mesenteric/splanchnic, pulmonary, renal, and skeletal muscle. Despite the increased baseline cardiac output, the ventricular contractile response to stimuli is attenuated, a condition termed cirrhotic cardiomyopathy (14). Other features of cirrhotic cardiomyopathy include blunted systolic and diastolic responses to stress, electrophysiological abnormalities, including prolongation of repolarization (increased electrocardiographic QT interval), and cardiac chamber hypertrophy or enlargement (8, 14).

Both conditions are clinically relevant: hyperdynamic circulation contributes to the pathogenesis of portal hypertension and ascites, whereas cirrhotic cardiomyopathy contributes to poor outcomes following stresses such as liver transplantation and may be involved in the genesis of hepatorenal syndrome (8).

Of the regional vascular beds, the splanchnic/mesenteric circulation shows the most significant degree of vasodilatation. Moreover, the vasodilatation in this vascular bed is thought to contribute to the pathogenesis of portal hypertension because of the increased portal venous inflow. Therefore, many studies have focused on this regional vascular bed.

The pathogenesis of hyperdynamic circulation and cirrhotic cardiomyopathy remain incompletely clarified. Several factors including central neural dysregulation (21) and humoral factors such as nitric oxide likely play a pathogenic role. Recently, endogenous cannabinoids or endocannabinoids have also emerged as a humoral/neural factor involved in the genesis of these circulatory abnormalities of cirrhosis.

Cannabinoids, both exogenous and endogenous, exert significant vascular and cardiac actions. The hemodynamic effects of cannabinoids are complex and vary significantly depending on experimental protocol and underlying autonomic tone (19). For example, the effects may vary according to cannabinoid or antagonist doses, vascular bed, animal species, anesthetic, or in vivo vs. in vitro preparations. Moreover, endocannabinoids such as arachidonylethanolamide (anandamide) exert vasoactivity not only through cannabinoid receptors CB1 and CB2 but also via vanilloid and other cannabinoid receptors that remain to be identified. Thus drawing firm conclusions about the hemodynamic effects of cannabinoids is difficult. However,
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Themes

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Despite the great variability and complexity in the cardiovascular responses, the consensus view is that cannabinoids are vasodilatory in the normal noncirrhotic animal or blood vessel.

This review focuses on the evidence of cannabinoid involvement in the vascular and cardiac changes of cirrhosis and portal hypertension. In addition to the caveats above, one must also bear in mind an “autocompensatory” phenomenon that affects in vivo studies of cannabinoids. The dominant vascular and cardiac effects tend to oppose each other: cannabinoids directly depress cardiac contractility via a CB1-mediated negative inotropism, whereas the peripheral vasodilation reduces cardiac afterload. Thus, in many in vivo studies, the overall effects on the circulation are minimal or undetectable. This is further complicated by the influence of cardiovascular reflexes that attempt to maintain circulatory homeostasis.

Although there is limited human data, most studies of the cardiovascular effects in cirrhosis have been performed using animal models. In particular, two rodent models of cirrhosis have been used in the vast majority of cannabinoid studies, the chronic bile duct-ligated (BDL) and the carbon tetrachloride-induced cirrhotic rat. Both models demonstrate the hemodynamic features of cirrhosis including portal hypertension and hyperdynamic circulation. The main differences between the two are the intense cholestasis and jaundice and greater extent of hepatocellular insufficiency in the BDL rat.

Two CB1 receptor antagonists have been predominantly used to explore cannabinoid physiology in cirrhosis, AM251 and SR141716A (rimonabant). Both antagonists have approximately equal affinity for the CB2 receptor, whereas the CB1 receptor is about 10 times more sensitive (15, 19).

Endocannabinoid Levels and Receptor Characteristics in Cirrhosis

There is little doubt that levels of anandamide are increased in cirrhosis. Batkai et al. (1) reported that monocytes isolated from cirrhotic patients and rats contained two- to threefold higher concentrations of anandamide compared with those of control samples. Injection of these monocytes to normal recipients significantly increased arterial blood pressure (1). In control rats, this drug slightly but significantly decreased blood pressure and thus rendered arterial pressures similar across the cirrhotic and control groups, i.e., eliminated the peripheral vasodilatation of cirrhosis. Ros et al. (20) also observed a pressor effect of SR141716A in the U46823-activated rat. In this study, cardiac output of cirrhotic rats was unaffected when measured at 10 and 30 min after drug injection (20). Rimonabant did not affect any hemodynamic variable in the control rats (20).

More recently, our laboratory showed a pressor effect of AM251 (3 mg/kg iv) in BDL-cirrhotic rats (16). The CB2 receptor antagonist AM630 did not affect any hemodynamic variable in either cirrhotic or control rats. In our study, following AM251 administration, cardiac output increased significantly at 5 min but then progressively decreased, reaching a significant nadir at 20 min, before gradually increasing back to control values by 30 min (16) (Fig. 1). Serial observation of cardiac output at 5-min intervals for 1 h demonstrated a variable time-dependent course and underscored the complexities involved in studying in vivo cannabinoid effects. Although we found, like Ros and colleagues (20), that cardiac

Systemic Hemodynamics

Cannabinoid receptor antagonist administration in cirrhotic rats. The vascular effect of endocannabinoids in cirrhosis was first reported by Bátkai and colleagues in 2001 (1). A single intravenous injection of SR141716A (3 mg/kg), in both rat models of cirrhosis (CCl4 and BDL) significantly increased arterial blood pressure (1). In control rats, this drug slightly but significantly decreased blood pressure and thus rendered arterial pressures similar across the cirrhotic and control groups, i.e., eliminated the peripheral vasodilatation of cirrhosis. Ros et al. (20) also observed a pressor effect of SR141716A in cirrhotic rats. In that study, cardiac output of cirrhotic rats was unaffected when measured at 10 and 30 min after drug injection (20). Rimonabant did not affect any hemodynamic variable in the control rats (20).

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Cardiac index (ml/min/100 g

Time (min)

Fig. 1. Cardiac output response to AM 251 (3 mg/kg iv) at time 0 (arrow) in ketamine/xylazine anesthetized sham-operated controls and bile duct-ligated (BDL) cirrhotic rats. Note the significant increase at 5 min followed by a progressive decline to a nadir at 20 min and return to baseline by 25 min in the cirrhotic rats, whereas the controls remain unaffected. [From Moezzi (16).]
output at 10 and 30 min after drug injection was not significantly different from the baseline value, the measurement of only these two time points would have caused the oversight of the significant increase at 5 min and the significant trough at 20 min postinfusion. Moreover, cardiac output is affected by many factors beside direct cardiac contractility, so it is at best only an indirect parameter to measure ventricular function. Nevertheless, the immediate increase in cardiac output with AM251 likely reflects a direct cardiotimbulent effect of CB1 receptor blockade and supports the notion of a direct negative-inotropic effect of endocannabinoids (11).

In contrast, Yang et al. (22) found no changes on arterial pressure in anesthetized BDL-cirrhotic rats after acute infusion of sequential doses of AM251 (1, 3, 5, 7 mg/kg). Speculative explanations for this discrepancy include differences in anesthetic and the possibility of reflexive adaptation in arterial pressure after exposure to sequential doses of AM251. Thus, while there is general agreement on the effects of acute CB1 blockade on hemodynamics in cirrhotic rats, the role of interplay among neural, cardiac, and vascular effects of this intervention needs to be further clarified.

**Anandamide administration in cirrhotic rats.** In anesthetized normal rats, anandamide induces a triphasic blood pressure response, consisting of (1) an initial transient hypotension probably related to transient receptor potential vanilloid-1 (TRPV1; also called VR1) receptors, (2) a pressor response of unclear origin, and (3) a delayed hypotension attributable to CB1 receptor activation.

Batkai et al. (1) administered anandamide intravenously (bolus of 4 mg/kg) in BDL-cirrhotic rats and noted an intact first phase, enhanced second phase, and absent third phase. In contrast, our laboratory observed in BDL-cirrhotic rats that received anandamide (3 mg/kg, intravenously infused over 4 min) an absent first phase, enhanced second phase, and a delayed third hypotensive phase (16). Possible explanations for the discrepancy in the first and third phases include differences in anandamide dose, mode of administration, anesthetic, and timing of observations. In our study, preadministration of AM251 before anandamide infusion eliminated the third phase, slightly blunted the enhanced second phase, and exerted no effect on the absent first phase. These results are consistent with the known effects of AM251, which blocks CB1 receptors but not TRPV1 receptors, and suggest the involvement of CB1 receptors in these anandamide effects.

**Splanchnic Hemodynamics**

**Cannabinoid receptor antagonist administration in cirrhotic rats.** Three in vivo studies in cirrhotic rats using cannabinoid receptor antagonists have reported consistent results, all showing reduced mesenteric blood flow with these drugs. Batkai et al. (1) observed that SR141716A injection decreased mesenteric arterial blood flow and portal pressure in CCl4-cirrhotic rats. Our laboratory showed that acute AM251 reduced superior mesenteric arterial flow in BDL-cirrhotic rats (16). Intravital microscopy revealed that AM251 significantly constricted mesenteric arterioles of cirrhotic rats but did not affect the venules or any blood vessel of control rats (16). Yang et al. (23) in BDL rats, examined acute and chronic (1 wk) AM251 administration. Again, the acute administration simultaneously decreased portal pressure and superior mesenteric arterial flow.

Chronic treatment with AM251 produced the same vascular effects in conjunction with decreases in hepatic collagen production, transforming growth factor-β, and several markers of the phospholipase A2 eicosanoid system (22). The portal hypotensive effect in that study may therefore have been partly mediated by the decreased liver fibrosis in addition to reduced portal venous inflow.

**Anandamide administration in cirrhotic rats.** Anandamide effects in the mesenteric circulation are also very concordant in all studies to date. In supraphysiological doses, it increases superior mesenteric arterial flow in cirrhotic rats without any significant effect in controls, an effect blocked by AM251 or SR141716 preadministration (16, 22). During anandamide infusion, BDL mesenteric venules also significantly dilate but less prominently than the arterioles. Anandamide has no significant effect on mesenteric arteriolar and venular diameters of control animals with or without AM251 preadministration (16). Although mesenteric veins share many of the same vasoactive and regulatory systems as arteries, they have generally been ignored in cardiovascular studies in cirrhosis because they are capacitance rather than resistance vessels. However, these results, as well as other studies showing blood volume pooling in the intrahepatic (12) and mesenteric veins (13), emphasize that gut veins and their capacitance function are important in overall cardiovascular regulation in cirrhosis.

Yang et al. (24) reported that anandamide induced a dose-dependent increase in portal perfusion pressure in isolated perfused livers of both normal and BDL-cirrhotic rats, but the magnitude of the effect was more pronounced in the latter group. Preincubation with indomethacin significantly attenuated the hyperresponsiveness of cirrhotic livers to anandamide but did not affect the responses in normal livers. There was a concomitant increase in thromboxane B2 and cysteinyl leukotrienes in the perfused liver, thereby suggesting that increased cyclooxygenase-derived eicosanoids might mediate this anandamide effect. Further studies by this group in isolated mesenteric arteries from BDL rats suggested that anandamide mediates its direct vasodilatory effects by activating vascular Ca2+ dependent potassium channels (24).

Anandamide-induced relaxation is also significantly potentiated in phenylephrine-precontracted mesenteric vascular beds of 7-day BDL rats (17). This is a model of cholestasis with some liver architectural disorganization but no cirrhosis. Chronic treatment of these BDL animals with nitro-l-arginine methyl ester (l-NNAME, a nonselective nitric oxide synthase inhibitor) and aminoguanidine [a selective inducible nitric oxide synthase (iNOS) inhibitor] blocked this hyperresponsiveness. Although acute l-NNAME treatment of mesenteric beds completely blocked the anandamide-induced vasorelaxation in sham-operated rats, this vasorelaxation was still present in the BDL rats. The divergent effects of acute vs. chronic NOS blockade thus makes it difficult to clarify the exact interrelationship of NO and endocannabinoids in the vasodilatation of cholestatic liver disease (17). Another study also demonstrated that isolated mesenteric arteries of CCl4-cirrhotic rats are more sensitive to anandamide than control vessels (5). However, anandamide did not affect femoral arteries in either group (5), emphasizing the regional vascular selectivity of endocannabinoids and suggesting these compounds as an important local regulator of mesenteric vascular tone in cirrhosis.
Vanilloid System

Anandamide and vanilloids share some structural similarities. In rat mesenteric arteries, the endothelium-independent vasodilator effect of anandamide is inhibited by the VR1 receptor antagonist capsazepine or by a calcitonin gene-related peptide (CGRP) receptor antagonist. Anandamide binds to a cloned VR1 receptor with micromolar affinity (4) and at nanomolar concentrations releases CGRP from sensory nerve terminals in the vascular adventitia (25). Thus it is likely that anandamide acts at VR1 receptors in sensory nerves to release the potent vasodilator peptide CGRP. Accordingly, any discussion of cannabinoid involvement in the hemodynamics of cirrhosis must also involve mention of the vanilloid system.

Two studies have examined the interaction of the vanilloid and cannabinoid systems in the hemodynamics of cirrhosis, with generally good agreement. Domenicali and colleagues (5) showed that, in the CCl4-cirrhotic rat mesenteric vessel, pretreatment with capsazepine significantly attenuates the relaxation induced by anandamide although to a lesser extent than that observed under CB1 receptor blockade. They also reported that simultaneous CB1 and TRPV1 receptor blockade almost completely prevents the anandamide-induced vasorelaxation (5). Our laboratory demonstrated that capsazepine does not significantly affect arterial pressure in BDL-cirrhotic or control rats (16). However, in cirrhotic rats, this drug reduces cardiac output and increases systemic vascular resistance. Capsazepine significantly constricts mesenteric arterioles of BDL rats but does not affect venules or any vessel diameters of control rats. Sequential combination of both AM251 and capsazepine administration in the same cirrhotic animal generally did not produce additive or incremental effects beyond those observed with each drug alone, regardless of which drug was administered first (16). Both studies observed increased VR1 receptor expression in mesenteric arteries of cirrhotic rats compared with controls (5, 16).

Because capsazepine administration does not affect arterial pressure but constricts mesenteric vessels, it can be surmised that vanilloid receptors exert relatively little or no tonic influence on blood pressure regulation but rather play a role in the mesenteric hyperemia of cirrhosis. It can be concluded that the elevated level of anandamide in cirrhotic animals mediates mesenteric vasodilation via both CB1 and VR1 receptors. Most of our understanding about interaction between endocannabinoid and vanilloid systems comes from in vitro and gene knockout studies. More in-depth in vivo investigations, especially chronic manipulation of vanilloid signaling, are needed, particularly to help develop potential therapies based on these systems.

Cannabinoids and Cardiac Dysfunction of Cirrhosis

Cardiac contractility. Endogenous and exogenous cannabinoids are known to have direct effects on the heart. It has been shown that anandamide dose dependently decreases the contractile force of electrically driven isolated normal rat heart (7). The negative effect of endocannabinoids on cardiac contractility is generally attributed to CB1 receptors (2, 9). However, Ford et al. (7), using several selective and less selective CB1, CB2, and VR1 receptor antagonists, suggested that these effects are mediated by novel receptors distinguished from CB1 and CB2. This intriguing notion needs further experimental evidence to support it.

The mechanisms underlying cirrhotic cardiomyopathy remain incompletely clarified. Given that cannabinoids are cardiovascular, it was logical to investigate their possible role in the pathogenesis of cirrhotic cardiomyopathy. The aforementioned studies by Batkai et al. (1) and Ros et al. (20) both reported normalization of hyperdynamic circulation by SR141716 in two cirrhotic rat models. At that time, this effect was purely attributed to the peripheral vascular actions of endocannabinoids.

Our laboratory performed in vitro studies on isolated left ventricular papillary muscles of BDL-cirrhotic rats. In vitro administration of the CB1 antagonist AM251 restored blunted cardiac responsiveness to β-adrenergic stimulation (9). This was the first evidence supporting a direct effect of endocannabinoids on cardiac contractile dysfunction in cirrhosis. The relatively selective CB2 antagonist AM630 did not significantly affect contractility (9). This study also provided pharmacological evidence of increased local production of endocannabinoids in the cirrhotic heart (9). Moezi et al. (16) provided the first in vivo evidence supporting a direct cardiac effect of AM251 in cirrhotic rats, showing an initial increased cardiac output after drug injection. The authors concluded that this initial increase was because of a direct action of AM251 on cardiac muscle (16).

Recently, Batkai et al. (2) performed a series of in vivo experiments on rats with CCl4-induced cirrhosis. They measured indexes of left ventricular contractility by in vivo left ventricular pressure-volume loop experiments. The pressure-volume loop allows assessment of ventricular contractility independent of the loading conditions; thus these findings further confirm a direct cardiac effect of AM251 separate from its peripheral vascular actions.

Two preliminary studies support the view that CB1 mediates negative inotropic effects in the cirrhotic heart. We provided in vivo left ventricular pressure-volume data that supported the same findings with similar time points (Fig. 2) (unpublished observations). It could be argued that in vivo effects of CB1 and other endocannabinoids acutely and relatively selectively affect cardiac contractility independent of the loading conditions. This concept is consistent with the notion that CB1 receptor antagonists such as AM251 and capsazepine do not significantly alter arterial pressure, heart rate, or cardiac output in control rats. This study therefore suggests that the cardiac remodeling associated with cirrhosis may result from a direct pharmacological effect of AM251 on cardiac muscle independent of loading conditions. Further studies are needed to confirm this direct cardiac effect of AM251 and to define the role of CB1 receptor antagonists in the cardiac remodeling associated with cirrhosis.
antagonists are mediated by their action on the autonomic nervous system. However, no significant alteration in the heart rate variability pattern was noted after administration of AM251 in cirrhotic rats (Moore KP, personal communication). This finding does not support modulation of cardiac autonomic tone by AM251, but this issue requires further investigation. Taken together, these studies strongly suggest that, in the cirrhotic heart, local overproduction of anandamide exerts a direct negative inotropic effect through CB1 receptors. The exact local origin of the increased anandamide remains unclear. It may be originating directly from cardiac myocytes, endothelial cells, or even infiltrating monocytes and macrophages. Because of the aforementioned autocompensation action of endocannabinoids, in vivo studies must be interpreted cautiously. Load-independent techniques such as the pressure-volume loop setups or isolated cardiomyocytes are necessary to elucidate the direct cardiac effects of cannabinoids.

**Cardioprotection.** There are several studies of cardioprotective actions of endocannabinoids. Lepicier et al. (11) reported a mainly CB2-mediated cardioprotective role for anandamide in a rat model of ischemia-reperfusion. Similarly, Lamontagne et al. (10) reported a CB2-mediated cardioprotective role in an animal model of myocardial ischemia. A study on mice lacking fatty acid amide hydrolase (FAAH, the enzyme that breaks down cannabinoids) showed a protective role for FAAH knockout against age-dependent decline in cardiac function (3). This cardioprotection was associated with a decline in myocardial gene expression of TNF, gp91phox, matrix metalloproteinase-2 (MMP-2), MMP-9, caspase-3 and -9, myocardial iNOS protein expression, nitrotyrosine formation, Poly ADP ribose polymerase cleavage, and caspase 3/9 activity.

Considering this evidence for a cardioprotective role of endocannabinoids, one might speculate on a similar role for local endocannabinoid overactivity in cirrhosis. To date only short-term effects of cannabinoid receptor antagonists have been studied, and the results show improvement of cardiac contractility, which could potentially result in better renal perfusion and reduced pulmonary congestion. However, investigating long-term effects of these antagonists is crucial to detect possible detrimental effects of removing a cardioprotective mechanism away from an already stressed heart.

**GRANTS**

Our studies mentioned in this review were funded by operating research grants from the Canadian Institutes of Health Research. S. Gaskari was supported by a Canadian Heart and Stroke Foundation trainee award.

**REFERENCES**


