LIVER

Increased anandamide induced relaxation in mesenteric arteries of cirrhotic rats: role of cannabinoid and vanilloid receptors

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Background and aims: Anandamide is an endocannabinoid that evokes hypotension by interaction with peripheral cannabinoid CB1 receptors and with the perivascular transient receptor potential vanilloid type 1 protein (TRPV1). As anandamide has been implicated in the vasodilated state in advanced cirrhosis, the study investigated whether the mesenteric bed from cirrhotic rats has an altered and selective vasodilator response to anandamide.

Methods: We assessed vascular sensitivity to anandamide, mRNA and protein expression of cannabinoid CB1 receptor and TRPV1 receptor, and the topographical distribution of cannabinoid CB1 receptors in resistance mesenteric arteries of cirrhotic and control rats.

Results: Mesenteric vessels of cirrhotic animals displayed greater sensitivity to anandamide than control vessels. This vasodilator response was reverted by CB1 or TRPV1 receptor blockade, but not after endothelium denudation or nitric oxide inhibition. Anandamide had no effect on distal femoral arteries. CB1 and TRPV1 receptor protein was higher in cirrhotic than in control vessels. Neither CB1 mRNA nor protein was detected in femoral arteries. Immunochemistry showed that CB1 receptors were mainly in the adventitia and in the endothelial monolayer, with higher expression observed in vessels of cirrhotic rats than in controls.

Conclusions: These results indicate that anandamide is a selective splanchnic vasodilator in cirrhosis which predominantly acts via interaction with two different types of receptors, CB1 and TRPV1 receptors, which are mainly located in perivascular sensory nerve terminals of the mesenteric resistance arteries of these animals.

The endogenous cannabinoid family includes anandamide (AEA), 2-arachydonyl glycerol, virodhamine, noladin-ether, and N-arachidonoyl-dopamine.[1] These substances elicit cardiovascular effects, such as hypotension, via activation of the cannabinoid CB1 receptor.[2] AEA also promotes vasodilation in some areas, such as the splanchnic vascular bed.[3] The CB1 receptor is peripherally located in endothelial and smooth muscle cells and in perivascular nerves.[2 4] Moreover, AEA can also interact with the transient receptor potential vanilloid type 1 protein (TRPV1), which is also known as VR1 receptor and belongs to the large family of TRP ion channels and is expressed in perivascular nerves.[4]

Recent studies have identified the endogenous cannabinoid system as a mediator involved in the pathogenesis of arterial hypotension in cirrhosis.[5 6] However, whereas the ability to produce increased amounts of endocannabinoids is clearly established in experimental and human cirrhosis, no study has assessed either vascular reactivity to AEA in cirrhotic resistance vessels or whether this response is differentially regulated depending on the vascular territory. Therefore, the aim of the current investigation was to assess whether mesenteric resistance arteries of cirrhotic rats with ascites display an altered and differential response to AEA. Our results demonstrated that this endocannabinoid produced a markedly enhanced vasorelaxant effect in mesenteric arteries of cirrhotic rats with ascites, a phenomenon not observed in the peripheral vasculature, mediated by both CB1 and TRPV1 receptors located mainly in perivascular nerves. These findings point to the endocannabinoid system as an important pathogenic mediator of splanchnic arteriolar vasodilation in advanced liver disease.

MATERIAL AND METHODS

The study was performed in cirrhotic adult male Wistar rats with ascites and in control Wistar rats (Charles-River, Saint Aubin les Elseuf, France). Cirrhosis was induced with CCl_4 following a method described previously.[7]

Isolation of femoral and mesenteric arteries was performed as described by Wigg and colleagues.[8] Briefly, rats aged 24–28 weeks were killed by decapitation and the mesenteric bed and femoral arteries were removed and placed in Krebs solution (mM): 120.0 NaCl, 5.0 KCl, 25.0 $NaHCO_3$, 11.0 glucose, 1.0 KH_2PO_4, 1.2 $MgSO_4$, and 2.5 $CaCl_2$, equilibrated with 5% CO_2–95% O_2. To asses vascular reactivity in resistance vessels, distal branches of the femoral artery and tertiary branches of the mesenteric arcades (both 200–300 μm external diameter) were dissected and segments (2 mm) were individually mounted on a small vessel wire myograph (Model 610M; JP Trading, Aarhus, Denmark). The integrity of the vessel was assessed on examination of the maximal force developed to 10 μM of the α_1 adrenoreceptor selective agonist phenylephrine and by subsequent determination of the effect of the endothelium dependent vasodilator acetylcholine (ACh 10 μM); relaxation ⩾80% was taken as indicating endothelium intact vessels. The preparations were allowed to recover for 30 minutes and were then primed

Abbreviations: TRPV1, transient receptor potential vanilloid type 1 protein; AEA, anandamide; ACh, acetylcholine; RT, reverse transcribed; PCR, polymerase chain reaction; L-NAME, Nω-nitro-L-arginine-methyl-ester; NO, nitric oxide; CGRP, calcitonin gene related peptide; CB receptor, cannabinoid receptor; pA2, apparent affinity dissociation constant; V_{max}, maximum response; SDS, sodium dodecyl sulphate three times over 30 minutes with phenylephrine (10 μM). Vessels were rinsed and thereafter Krebs solution contained indomethacin (1 μM) to prevent the influence of cyclooxygenase derived products throughout the subsequent experiments. Concentration-response curves to AEA were performed in mesenteric resistance arteries from cirrhotic and control rats precontracted with 10 μM phenylephrine. In preliminary experiments we found that 10 μM phenylephrine produced a similar constrictor response in mesenteric vessels of cirrhotic (9.68 (1.55) mN; n = 7) and control (9.81 (1.49) mN; n = 6) rats.

When indicated, endothelium denuded vessels were obtained by gently rubbing the intimal surface with a human hair. Vessels were considered endothelium denuded when ACh induced relaxation was less than 10% of precontracted tone.

Phenylephrine (10 μM) precontracted resistance arteries isolated from cirrhotic and control rats were exposed to cumulative concentrations of AEA, ranging from 10^{-8} to 5×10^{-4}M, in a wire myograph with four parallel independent chambers to evaluate the vessel response from the same rat under different experimental conditions. Firstly, the vasorelaxant effect of AEA was assessed. Next, concentration-response curves to AEA were performed in endothelium denuded arteries or in intact arteries in the absence or presence of the nitric oxide synthase inhibitor Nω-nitro-L-arginine-methyl-ester (L-NAME 100 μM) and the neurotoxin capsaicin (10 μM for one hour), respectively. The vasorelaxant effect of AEA under conditions of CB1 receptor blockade with SR141716A (3 μM) or TRPV1 receptor blockade with capsazepine (5 μM), alone or in combination, was evaluated. Finally, femoral resistance arteries isolated from cirrhotic and control rats were exposed to cumulative doses of AEA, as described above.

CB1 and TRPV1 mRNA expression in mesenteric and femoral arteries of control and cirrhotic rats

Total RNA was extracted from second and third order mesenteric arteries and distal femoral arteries from control and cirrhotic rats, and brain from control rats using a commercially available kit (Trizol Reagent; Life Technologies, Gaithersburg, Maryland, USA). Total RNA (0.5 μg) was reverse transcribed (RT) using a complementary DNA synthesis kit (Promega, Madison, Wisconsin, USA). Primers for CB1 receptor (sense: 5′-TGT GGG CAG CCT GTT CCT CA-3′; antisense: 5′-GGG TTT TGG CCA GCC TAA TGT C-3′) and for TRPV1 (sense: 5′-GGA CGT TGC CCG GAA GAC AGA C-3′; antisense: 5′-CTT CAG CGT GGG GTG GAG TTT G-3′) were prepared according to the rat CB1 mRNA sequence (GenBank accession No NM_012784) and the rat TRPV1 gene sequence (GenBank accession No AF327067), respectively. Polymerase chain reaction (PCR) for CB1 and TRPV1 were performed using a DNA amplification kit (Life Technologies). PCR products were sequenced to check correct amplification.

CB1 and TRPV1 protein expression in mesenteric and femoral arteries of control and cirrhotic rats

Distal branches of the femoral artery and tertiary branches of the mesenteric artery from control and cirrhotic rats were homogenised in a Tris HCl 20 mM pH 7.4 buffer, containing 1% Triton X-100, 0.1% sodium dodecyl sulphate (SDS), 50 mM NaCl, 2.5 mM EDTA, 1 mM $Na_4P_2O_7\cdot10H_2O$, 20 mM NaF, 1 mM Na_3VO_4, 2 mM Pefabloc, and a cocktail of protease inhibitors (Complete Mini; Roche, Basel, Switzerland). To detect the CB1 receptor, 80 μg of the denatured proteins were run on a 7.5% SDS-polyacrylamide gel and transferred to nitrocellulose membranes which were blocked with 5% powdered defatted milk in TTBS buffer (50 mM Tris-HCl, pH 8, containing 0.05% Tween 20 and 150 mM NaCl) overnight at 4℃, and were then incubated with a primary rabbit polyclonal antibody against the CB1 receptor (1:750; Cayman Chemical, Ann Arbor, Michigan, USA), followed by incubation with horseradish peroxidase conjugated rabbit antibody (1:5000; Amersham, Little Chalfont, Buckinghamshire, UK). The denatured proteins (80 μg) were run on a 10% SDS-polyacrylamide gel and transferred to nitrocellulose membranes, blocked with 2% bovine serum albumin in TTBS buffer (50 mM Tris-HCl, pH 8, containing 0.05% Tween 20 and 150 mM NaCl), and then incubated with a primary rabbit polyclonal antibody against the capsaicin receptor (1:750, TRPV1; Chemicon, Temecula, California, USA), followed by incubation with horseradish peroxidase conjugated rabbit antibody (1:2000; Amersham). Bands were visualised by chemiluminescence (ECL western blotting analysis system; Amersham). Relative expression of CB1 and vanilloid receptors was determined by densitometric scanning (Phoretix International Ltd, Newcastle, UK).

Immunohistochemical localisation of CB1 receptor

Third order branches of the mesenteric artery from control and cirrhotic rats were fixed with 4% phosphate buffered paraformaldehyde (one hour) and included in OCT. Cross sections (14 μm) were cut onto gelatin coated slides. Sections were incubated with a primary rabbit polyclonal antibody against the CB1 receptor (1:50; Cayman Chemical) in phosphate buffered saline containing 2% bovine serum albumin for one hour at 37℃. After washing, rings were incubated with the secondary antibody, a donkey antirabbit IgG conjugated to Cy3 (1:200; Jackson Immuno Research Laboratories, West Grove, Pennsylvania, USA), for a further one hour at 37℃. Immunofluorescent signals were viewed with a confocal microscope (Leica TCS SP2) with a $\times40_{oil}$ objective. Whole mesenteric resistance arteries from cirrhotic and control rats were processed as described above for CB1 receptor localisation in perivascular sensory nerves.

Specificity of the immunostaining was evaluated by omission of the primary antibody and processed. Under these conditions, no staining was observed in the vessel wall of either control or cirrhotic rats.

Statistical analysis

Relaxation was expressed as a percentage of the contraction induced by phenylephrine. EC_{50} was defined as the concentration of agonist effective in producing a response which was 50% of the maximum response (V_{max}), and pEC_{50} corresponds to the negative decimal logarithm of EC_{50}.

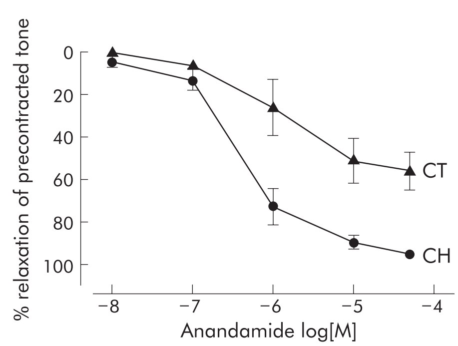

Figure 1 Anandamide induced relaxation in mesenteric arteries from cirrhotic and control rats: role of cannabinoid CB1 receptor. Log concentration-response curves for relaxation to anandamide of phenylephrine (10 μM) precontracted intact mesenteric arteries of cirrhotic (CH, n = 7) and control (CT, n = 6) rats; n indicates the number of rats used in each condition. $p<0.001$, CH versus CT (two way ANOVA).

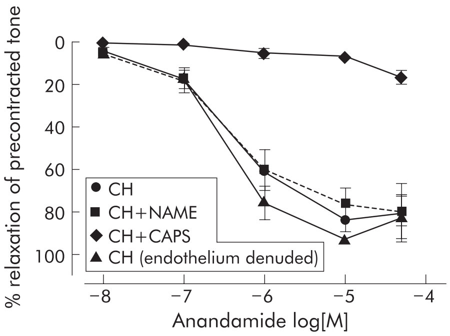

Figure 2 Anandamide induced relaxation in mesenteric arteries from cirrhotic rats: role of endothelium and nitric oxide. Log concentration-response curves for relaxation to anandamide in phenylephrine (10 μM) precontracted intact mesenteric arteries (cirrhotic rats (CH), n = 7), mesenteric arteries incubated with L-NAME (100 μM, n = 10), endothelium denuded mesenteric arteries (n = 5), and mesenteric arteries incubated with capsaicin (CAPS 10 μM, n = 8) of cirrhotic rats. n indicates the number of rats used in each condition. $p<0.001$, CH+CAPS versus all other conditions (two way ANOVA).

Estimated pA_2 was obtained from the equation, $pA_2 = \log((EC_{50A}/EC_{50C}) - 1) - \log[\text{antagonist}]$, where EC_{50A} is the agonist EC_{50} in the presence of antagonist and EC_{50C} is the control agonist of EC_{50}. Statistical comparisons of concentration-response curves were made by two way ANOVA of the whole data set, followed by Bonferroni's test for determining significant differences between treatment groups. Differences between pEC_{50} values or maximum relaxation were assessed by the unpaired Student's *t* test. Data are expressed as mean (SEM) and were considered significant at a p level of 0.05 or less. The study was performed according to the criteria of the Investigation and Ethics Committee of the Hospital Clínic Universitari.

RESULTS

Histological examination of livers from all animals treated with CCl_4 showed a finally granulated surface and the characteristic features of cirrhosis.

AEA induced a concentration dependent relaxation of endothelium intact, phenylephrine precontracted isolated mesenteric arteries from both cirrhotic and control rats at doses ranging from 10^{-8} M to 5×10^{-5} M (fig 1). The concentration response to AEA was shifted to the left ($p<0.001$). The maximal response to AEA (V_{max}) was approximately twofold higher in cirrhotic than in control arteries (93.14 (1.5)% (n = 7) *v* 56.41 (9.5)% (n = 6), respectively; $p<0,01$).

Additional experiments were conducted in intact, in endothelium denuded, and in intact L-NAME pretreated mesenteric arteries of cirrhotic rats. As shown in fig 2, no differences were observed in the response to AEA among the three experimental conditions. Increasing doses of the endocannabinoid resulted in similar values for both V_{max} (93.5 (1.6)% in intact vessels; 91.9 (1)% in endothelium denuded arteries; and 92.5 (6.9)% in L-NAME pretreated vessels) and pEC_{50} (6.4 (0.13); 6.45 (0.14); and 5.83 (0.33), respectively), indicating that the relaxation induced by AEA in cirrhotic vessels is not dependent on the functional integrity of the endothelium dependent NO metabolic pathway.

Figure 2 also shows the results obtained at preincubaiting isolated cirrhotic arteries with capsaicin, a selective neurotoxin for C fibres that results in functional desensitisation of the receptor system in perivascular nerves. Capsaicin pretreatment completely prevented the vasodilation induced by AEA in cirrhotic vessels, providing functional evidence that receptors located in the perivascular nerves are involved in the vascular effect of this cannabinoid.

Effect of CB1 and TRPV1 receptor blockade in mesenteric arteries of cirrhotic rats

To ascertain the contributory role of cannabinoid and vanilloid receptors in the vasorelaxant effect of AEA in mesenteric arteries of cirrhotic rats, vascular reactivity assays were also conducted under conditions of CB1 and TRPV1 receptor blockade (fig 3). AEA produced a concentration dependent relaxation of phenylephrine precontracted mesenteric arteries ($pEC_{50} = 6.9$ (0.23); $V_{max} = 96.8$ (0.8)%; n = 6). The CB1 receptor antagonist SR141716A (3 μM) shifted the concentration response curve to AEA to the right ($p<0.01$), with no significant reduction in maximal relaxation ($pEC_{50} =$ 5.30 (0.28); $V_{max} = 80.6$ (7)%; n = 6). Moreover, incubation with the TRPV1 antagonist capsazepine also shifted the concentration response curve to AEA ($pEC_{50} = 6.15$ (0.23), n = 4) to the right ($p<0.05$) with no modification in maximum relaxation (84.9 (14)%). Estimation of the magnitude of the shifts from the EC_{50} values gave a 44.7-fold shift for SR141716A and a 6.45-fold shift for capsazepine, which correspond to estimated pA2 values of 7.17 for SR 141716A and 6.04 for capsazepine. When both antagonists were incubated together, there was stronger inhibition of the response to AEA at the concentrations used. Nevertheless, the limited solubility of AEA

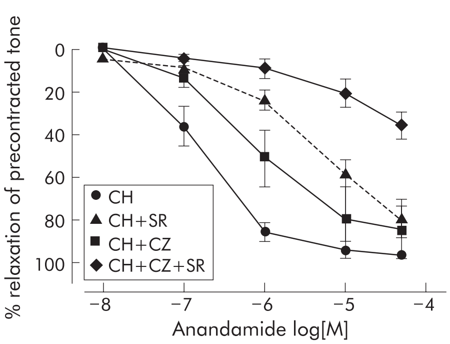

Figure 3 Anandamide induced relaxation in mesenteric arteries from cirrhotic rats: role of cannabinoid CB1 and transient receptor potential vanilloid type 1 protein (TRPV1) receptors. Log concentration-response curves for relaxation to AEA in phenylephrine (10 μM) precontracted intact mesenteric arteries (cirrhotic rats (CH), n = 6), mesenteric arteries incubated with SR141716A (SR 3 μM, n = 6), mesenteric arteries incubated with capsazepine (CZ 5 μM, n = 4), and mesenteric arteries incubated with SR141716A plus capsazepine (CZ+SR, n = 5) of cirrhotic rats. n indicates the number of rats used in each condition. $p<0.01$, CH versus CH+SR and CH versus CH+CZ+SR; $p<0.05$, CH versus CH+CZ (two way ANOVA).

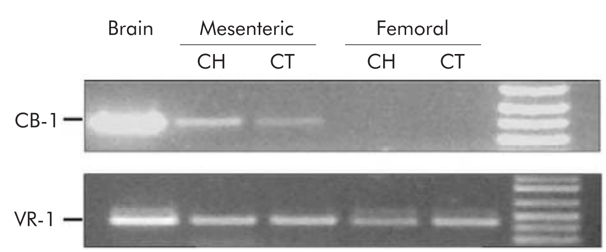

Figure 4 mRNA expression of cannabinoid CB1 and transient receptor potential vanilloid type 1 protein (TRPV1) receptors in mesenteric and femoral arteries of control (CT) and cirrhotic (CH) rats. Representative reverse transcription-polymerase chain reaction of mRNA for CB1 and TRPV1 (VR-1) receptor in second and third order mesenteric arteries and distal femoral arteries of control and cirrhotic rats is shown.

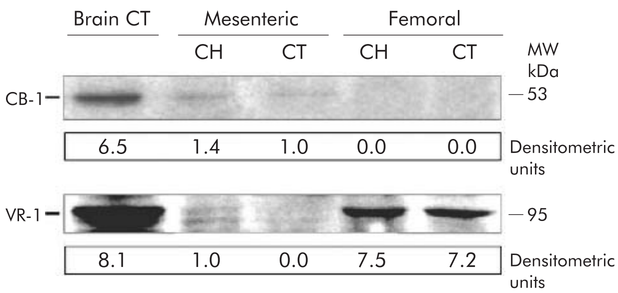

Figure 5 Western blot analysis of cannabinoid CB1 and transient receptor potential vanilloid type 1 (TRPV1) protein in mesenteric and femoral arteries of control (CT) and cirrhotic (CH) rats. A representative western blot of the CB1 receptor and the TRPV1 receptor in mesenteric and femoral arteries of control and cirrhotic rats with ascites is shown. Densitometric analysis of the representative western blots is shown at the bottom of the panels.

precluded the use of higher concentrations and the construction of a full concentration response curve.

As shown in fig 3, vessel pretreatment with the TRPV1 receptor antagonist capsazepine also significantly attenuated the relaxation induced by the endocannabinoid although to a lesser extent than that observed under conditions of CB1 receptor blockade. Finally, simultaneous CB1 and TRPV1 receptor blockade almost completely prevented the AEA induced relaxation in isolated mesenteric arteries of cirrhotic rats.

In addition, AEA had no vasodilatatory effect on distal femoral arteries of cirrhotic and control rats, indicating the existence of a selective action of the cannabinoid on splanchnic circulation. Similar results were observed in isolated vessels of control rats (data not shown).

CB1 and TRPV1 mRNA expression

As shown in fig 4, second and third order mesenteric arteries, from both control and cirrhotic rats, expressed a band of 425 bp, corresponding to the CB1 receptor mRNA. This band was absent in samples from distal femoral arteries, which is in agreement with the lack of response to AEA observed in this type of vessel. In contrast, both mesenteric and femoral arteries expressed mRNA for TRPV1, as reflected by amplification of a band of 442 bp in all samples.

CB1 and TRPV1 protein expression

Mesenteric arteries of cirrhotic and control rats showed a specific band of approximately 53 kDa that was identified as CB1 protein due to its correspondence with the band obtained in the positive control, thus providing evidence of antibody specificity (fig 5). Paralleling the results obtained on analysing CB1 PCR products, enhanced abundance of CB1 protein was detected in cirrhotic mesenteric arteries compared with controls, and no protein expression of this receptor was observed in the femoral arteries of both groups of animals. Western blot analysis of TRPV1 receptor yielded a specific band at the expected molecular weight of approximately 95 kDa in the positive control. In contrast with strong TRPV1 expression found in femoral arteries of cirrhotic and control rats, a faint signal was detected in cirrhotic mesenteric vessels and no detectable TRPV1 protein was observed in mesenteric vessels isolated from control rats (fig 5).

Immunodetection of CB1 receptors in mesenteric vessels

Confocal microscopy analyses were performed to unveil CB1 receptors in the different layers of mesenteric vessels. Figure 6

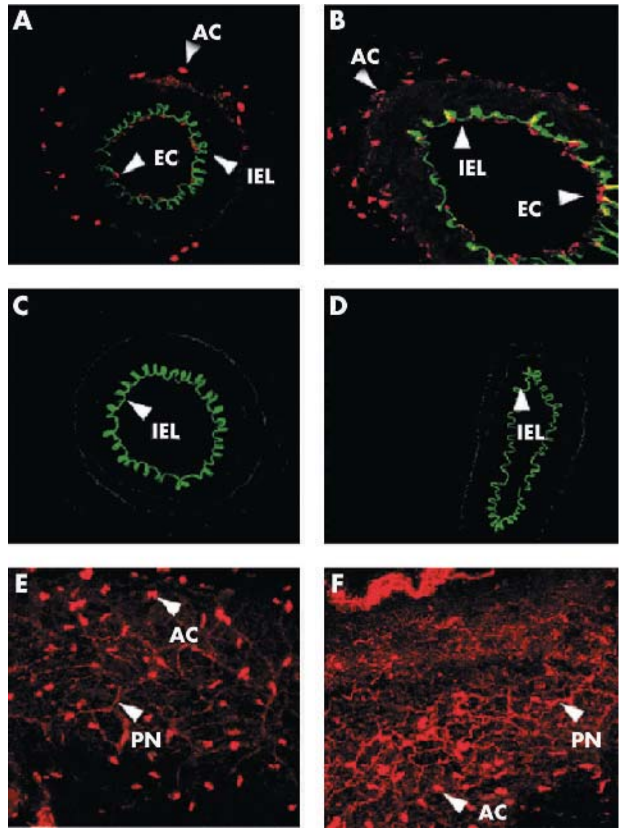

Figure 6 Immunodetection of cannabinoid CB1 receptors in mesenteric vessels. Representative confocal imaging of CB1 receptor in sections of mesenteric resistance arteries of control (A) and cirrhotic rats with ascites (B). (C, D) Negative controls of the fluorescence produced by omission of the primary antibody in arteries from the same animals. Fluorescence in whole mounted vessels of control (E) and cirrhotic (F) rats is also shown. Natural autofluorescence of the elastic components of the arterial wall are also depicted (n = 5). Image dimensions 256×256 μm. AC, adventitial cells; EC, endothelial cells; IEL, internal elastic lamina; PN, perivascular nerves.

shows that CB1 receptor (red) was mostly present in adventitial cells and only slightly in endothelial cells, in both control and cirrhotic mesenteric vessels. As shown, CB1 receptor density in adventitial cells from cirrhotic vessels (fig 6B) was higher than that in control vessels (fig 6A). These findings were in accordance with the response to AEA observed in these vessels. No expression of CB1 receptor was detected in the smooth muscle cell layer. Negative (fig 6C, D) control slides of control and cirrhotic mesenteric resistance vessels showed no staining.

To determine whether the CB1 receptor is expressed in perivascular sensory nerves innervating mesenteric resistance arteries, fluorescent immunohistochemistry in whole mounted vessels combined with confocal microscopy was also performed. Confocal microscopy showed that perivascular sensory nerves innervating mesenteric resistance arteries from control and cirrhotic rats were positively stained for CB1 (fig 6E, 6F). No staining was observed in arteries from the same animals in the absence of primary antibody (data not shown).

DISCUSSION

The main aim of the present study was to determine whether enhanced vascular reactivity to AEA selectively occurred in the mesenteric resistance vessels of cirrhotic rats. The results indicate that AEA is an important specific splanchnic vasodilator acting predominantly in a non-endothelium, non-NO dependent manner via interaction with two different types of receptors, CB1 and TRPV1 receptors, which are mainly located in perivascular sensory nerve terminals of the mesenteric resistance arteries of cirrhotic rats.

In terms of vasorelaxation to AEA, we have shown that isolated mesenteric resistance arteries of cirrhotic rats with ascites relaxed to a greater extent than vessels of control rats. Because previous investigations have also found increased circulating levels of AEA in cirrhosis,[5 6] our results indicate that the endocannabinoid system may have greater local vasodilator activity in the splanchnic circulation under this condition. In agreement with previous investigations in mesenteric arterial preparations,[9] our results showed that L-NAME had no effect on the pronounced relaxation induced by AEA in the mesenteric vessels of cirrhotic animals, suggesting that endothelium derived NO does not play a major role in this response. Consistent with the lack of effect of L-NAME, endothelial denudation also did not alter the vasorelaxation induced by the endocannabinoid in cirrhotic vessels, indicating that the effect of AEA on mesenteric vessels is not dependent on functional endothelium integrity. This is in keeping with several previous studies suggesting that the relaxation induced by AEA is largely independent of endothelium components.[9–11] CB1 receptors have been described in the perivascular sensory nerves of mesenteric arteries.[12] Moreover, several studies have also indicated that AEA may exert its vasorelaxant activity by stimulating CGRP release from sensory nerves through activation of TRPV1 receptors.[13] In this regard, recent studies have shown that neonatal capsaicin treatment blocks the development of the hyperdynamic circulation and ascites formation in bile duct ligated cirrhotic rats.[14] Therefore, to elucidate whether a perivascular rather than an endothelial component could be involved in the increased AEA induced vasorelaxation of the cirrhotic mesenteric arteries, experiments were also performed in the presence of capsaicin. This substance activates primary sensory nerves that then become refractory to subsequent stimuli because of desensitisation and/or neurotransmitter depletion.[15] Pretreatment with capsaicin fully abolished the AEA induced relaxation in resistance mesenteric arteries of cirrhotic rats, providing functional evidence that the signalling pathway ultimately resulting in vasorelaxation of cirrhotic mesenteric arteries is mainly located in the adventitia, rather than in the endothelial layer of the vessel.

The involvement of cannabinoid and/or vanilloid receptors in the vasorelaxation to AEA in mesenteric cirrhotic vessels was examined using both SR141716A and capsazepine. The CB1 receptor antagonist produced a marked rightward shift in the response curve to AEA without affecting the maximal response produced by the endocannabinoid. These results, which are coincident with previous investigations by Randall and colleagues,[16] favour the involvement of CB1 receptors. The fact that capsaicin inhibited responses to AEA together with the shift to the right of the concentration response curve to AEA by capsazepine could be explained by its ability to stimulate CGRP release from capsaicin sensitive sensory nerves as a consequence of activation of vanilloid TRPV1 receptors.[13] Interestingly, simultaneous blockade of CB1 and TRPV1 receptors almost fully prevented the vasorelaxant effect of AEA in cirrhotic vessels which is a strong additional indication that both CB1 and TRPV1 receptors are involved in the vasorelaxant effect of AEA in the mesenteric resistance vessels of cirrhotic rats.

Previous investigations have indicated that vasorelaxation induced by AEA is not a general phenomenon occurring in all vascular areas. This endocannabinoid has been shown to cause cerebrovascular, mesenteric, and coronary dilatation in the rat,[16–18] but not in the aorta or carotid artery.[19 20] This was also confirmed in the present investigation as no vascular response to AEA was observed in femoral resistance arteries of cirrhotic and control rats. Our results emphasise the tissue selectivity of endocannabinoids and point to AEA as an important local regulator of vascular tonicity in the mesenteric circulation in pathological conditions such as hepatic cirrhosis.

To further confirm the presence of CB1 receptor mRNA and protein in resistance mesenteric arteries, RT-PCR and western blot experiments were performed. To our knowledge, this is the first study demonstrating CB1 mRNA expression in third order mesenteric arteries of adult rats, with CB1 transcript expression being higher in cirrhotic than in control animals. Furthermore, no mRNA expression was detected in femoral arteries, irrespective of whether they were obtained from cirrhotic or control rats. Western blot experiments yielded parallel results to those found in the RT-PCR analyses. In both brain and mesenteric arteries, the CB1 specific antibody recognised the CB1 protein, while in femoral arteries CB1 protein expression was absent. Moreover, receptor signal intensity was higher in protein extracts obtained from mesenteric vessels of cirrhotic animals than in those of control animals. These findings coincide with those obtained in the vascular reactivity assays and suggest a major involvement of the CB1 receptor in the increased vascular responsiveness to AEA detected in the mesenteric arteries of cirrhotic rats.

The results of TRPV1 expression analysis were less conclusive than those for the CB1 receptor. A PCR product of the expected size was amplified for rat brain and mesenteric and femoral arteries, with no distinctive pattern expression between cirrhotic and control samples. As the amplified products encode for the functional receptor protein, we thereafter verified transcription of this mRNA into protein by western blot. An immunoreactive protein at approximately 96 kDa was identified in protein extracts from brain and femoral and mesenteric arteries although expression in the latter was very weak in comparison with the other tissues. This clearly differs from the vascular reactivity experiments in femoral arteries. The lack of response to AEA in femoral arteries despite the existence of TRPV1 receptors could be the consequence of the experimental conditions used in the current experiments but also could be due to the particular characteristics of this receptor. TRPV1 is a non-selective cation channel that integrates noxious stimuli and can also be activated by AEA.[15 21] However, significant differences have been described in the efficacy of AEA between different tissues.[22] Differences in efficacy of AEA at the TRPV1 in different tissues may result from several factors, the most important being AEA membrane transporter and fatty acid amide hydrolase activities.[15 23] In mesenteric arteries however, our functional studies together with the vascular expression assays indicate the existence of a TRPV1 component. Therefore, it is likely that, in addition to activating the CB1 receptor in resistance mesenteric arteries, AEA may also serve as an endogenous mesenteric TRPV1 stimulator further contributing to the splanchnic vasorelaxation occurring in advanced liver disease.

TRPV1 receptors are almost exclusively expressed in primary sensory neurones that surround and innervate resistance mesenteric arteries.[24] However, the CB1 receptor is more widely expressed among the different artery cell types, including endothelial and vascular smooth muscle cells[2 4] and also in perivascular nerves.[4] Therefore, we studied CB1 cellular distribution within cirrhotic and control mesenteric arteries using immunohistochemistry. Specific CB1 staining was detectable in endothelial and adventitial cells. We also found that CB1 receptor immunoreactivity was more intense in adventitial cells of cirrhotic vessels than in those of controls. This pattern of staining is consistent with the increased CB1 mRNA and protein expression observed in cirrhotic arteries and supports the results of the vascular reactivity assays showing an endothelium independent increased vasorelaxant response to AEA in mesenteric arterial vessels of cirrhotic rats.

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Conflict of interest: None declared

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