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Potentiation of anandamide effects in mesenteric beds isolated from bile duct-ligated rats: role of nitric oxide

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Abstract

Changes in vascular responsiveness are proposed as the basis for some of the cardiovascular complications in cholestasis. Cholestasis is also associated with accumulation of endogenous opioid peptides and evidence of nitric oxide (NO) overproduction. On the other hand, it is well known that anandamide, an endogenous cannabinoid ligand, causes hypotension and a decrease in systemic vascular resistance. In the present study, the possible role of the cannabinoid system in cholestasis-induced mesenteric vascular bed responsiveness was investigated. Mesenteric arteries of bile duct-ligated and sham-operated rats receiving daily administrations of saline were used for evaluating phenylephrine or anandamide dose–response, acute effects of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM), a non-selective inhibitor of NO synthase (NOS), or naltrexone, an opioid receptors antagonist (1 μM). The other groups of bile duct-ligated and sham-operated rats received daily intraperitoneal administration of L-NAME (20 mg/kg/day), aminoguanidine, a selective inducible NOS (iNOS) inhibitor (150 mg/kg/day) or naltrexone (10 mg/kg/day). After 7 days, the superior mesenteric artery was cannulated and the mesenteric vascular bed was perfused according to the McGregor method. Anandamide-induced relaxation was significantly potentiated in mesenteric vascular beds of bile duct-ligated rats. Chronic treatment of bile duct-ligated animals with L-NAME and aminoguanidine blocked this hyperresponsiveness while the hyperresponsiveness was potentiated at large doses of anandamide on chronic treatment of these animals with naltrexone. Although acute L-NAME treatment of mesenteric beds completely blocked the anandamide-induced vasorelaxation in sham-operated rats, this vasorelaxation still was present in bile duct-ligated animals. Anandamide-induced vasorelaxation remained unaffected after acute naltrexone treatment of mesenteric beds in both bile duct-ligated and sham-operated rats. Our results indicate that (1) there is enhanced anandamide-induced vasorelaxation in cholestatic rats, probably due to a defect in cannabinoid or vanilloid receptors and (2) NO overproduction may be involved in cholestasis-induced vascular hyperresponsiveness.

Keywords: Cholestasis; Cannabinoid system; Anandamide; Nitric oxide (NO); Opioid system; Mesenteric vessel; (Rat)

1. Introduction

Cholestasis is associated with aberrations in cardiovascular function typified by a tendency to hypotension and renal failure (Dabagh et al., 1999; Bomzon et al., 1996). The exact etiology of these problems is elusive, but vascular hyporesponsiveness to sympathetic stimulation (Bomzon et al., 1985; Jacob et al., 1993) is thought to play an important role. A number of factors such as bile salts (Pak and Lee, 1993), endotoxin (Inan et al., 1997) and elevated level of prostaglandins (Cioffi et al., 1986) are proposed for cholestatic-induced vascular hyporesponsiveness.

Evidence has been reported for nitric oxide (NO) overproduction and elevated plasma levels of endogenous opioid peptides in cholestasis (Nahavandi et al., 1999, 2000; Mani et al., 2001, 2002; Thornton and Lowosky, 1988). There is also increasing evidence for roles of NO and endogenous opioids in the pathophysiology and manifestations of cholestasis including vascular hyporesponsiveness (Dehpour et al., 1999; Jones and Bergasa, 2000; Kimpel et al., 1998; Namiranian et al., 2001).

Endogenous cannabinoids represent a novel class of lipid ligands that share receptor binding sites with plant-derived cannabinoids, such as Δ^9-tetrahydrocannabinol (Wagner et

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al., 1999). To date, two cannabinoid receptors have been cloned, the cannabinoid CB_1 receptor expressed primarily in the brain (Matsuda et al., 1990), but also in some peripheral tissues (Varga et al., 1996; Shire et al., 1995) and the cannabinoid CB_2 receptor expressed by cells of the immune system (Munro et al., 1993). In addition to having their well-known neurobehavioral effects, anandamide, the endogenous ligand of cannabinoid receptors and Δ^9-tetrahydrocannabinol influence a number of other physiological functions, including cardiovascular variables (Jarai et al., 1999). Anandamide has been shown to be a vasorelaxant, particularly in the resistance vasculature. This vasorelaxation has been reported to be both endothelium-independent and -dependent, depending on the vascular bed (Randall and Kendall, 1998).

On the other hand, it has been shown that *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamidehydrochloride (SR141716A), a cannabinoid receptor antagonist, is able to elevate the blood pressure of cirrhotic rats. Monocytes of cirrhotic patients had increased level of anandamide and a three-fold increase in cannabinoid CB_1 receptors has been seen in cirrhotic human vascular endothelial cells (Batkai et al., 2001).

We investigated the possibility of anandamide vasorelaxation changes in mesenteric vascular beds of bile duct-ligated rats. We further evaluated the roles of nitric oxide and opioid systems in the probable anandamide-induced vasorelaxation disturbances.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 200–250 g were used in this study. All animals were given free access to food and water. The animals were handled in accordance with the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" (NIH US publication 86-23 revised 1985). The animals were randomly divided into 14 groups; each group consisted of 6–7 rats. Seven groups were sham-operated and another seven groups underwent bile duct ligation. Bile duct ligation was performed as described previously (Nahavandi et al., 1999). Laparatomy was performed under anesthesia (ketamine HCl, 50 mg/kg, i.p. and promazine HCl, 10 mg/kg, i.p.). In the sham-operated rats, the bile duct was identified, manipulated and one untied loose tie was left in place, but in bile duct-ligated rats, the bile duct was doubly ligated. Then the abdominal wall was closed in two layers.

Four groups of bile duct-ligated and sham-operated rats were treated with daily intraperitoneal administration of isotonic sterile saline solution (10 ml/kg/day), two groups serving as control groups for phenylephrine or anandamide dose–response and the other two for in vitro acute effects of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM), a non-selective inhibitor of nitric oxide synthase (NOS), or naltrexone (1 μM), an opioid receptors antagonist, in mesenteric vascular beds.

The last three groups of sham-operated and bile duct-ligated rats received daily intraperitoneal administration of L-NAME (20 mg/kg/day), aminoguanidine, a selective inducible NOS (iNOS) inhibitor (150 mg/kg/day) or naltrexone (10 mg/kg/day). These rats received seven doses of the above-mentioned drugs for 7 consecutive days. The first dose was injected after the surgery and the last dose was injected 24 h before killing.

2.2. Bilirubin measurement

A sample (3 ml) of blood was collected at the time of killing and bilirubin was determined with a commercially available kit (Zistshimi, Tehran, Iran).

2.3. Preparation of mesenteric vascular bed

After 7 days, the rats were anesthetized with ether and the mesenteric vascular bed was prepared as described by McGregor (1965). The abdominal wall was opened and the superior mesenteric artery was identified, cannulated and gently flushed with modified Krebs–Henseleit solution (containing (mM) NaCl: 118, KCl: 4.7, $CaCl_2$: 2.5, $MgSO_4$: 1.2, dextrose: 11, $NaHCO_3$: 25, NaH_2PO_4: 1.2), which was bubbled with a mixture of 95% O_2 and 5% CO_2 (final pH: 7.4) and warmed to 37 °C before it entered the pump. After 5 min of perfusion with 2 ml/min, the mesentery was separated from intestine by cutting close to the intestinal border of the mesentery. Only the main arterial branches from the superior mesenteric artery running to terminal ileum were perfused. Then, the rate of perfusion was increased to 5 ml/min. The tissue was prevented from drying by hyperperfusion with 0.5

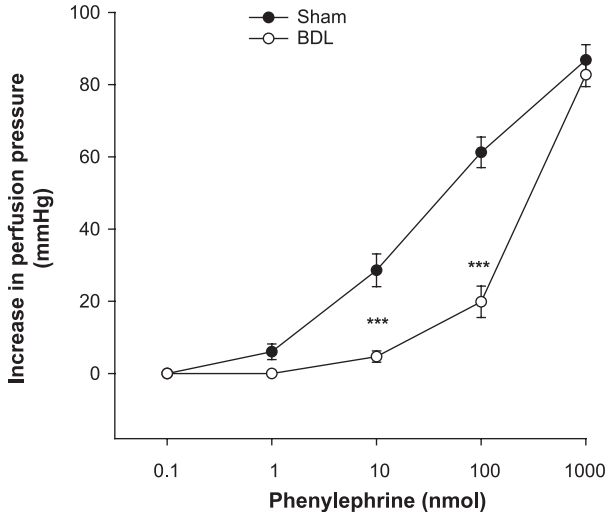

Fig. 1. The vasoconstriction response curve to phenylephrine for the mesenteric vascular bed of sham-operated and bile duct-ligated rats. Each point represents the mean ± S.E.M. for six to seven rats. ***$P < 0.001$ compared with the corresponding sham values.

ml/min solution and was warmed by placing it on a constant temperature (37 °C) bath.

A peristaltic pump (Pump speed control Model 500–1200, Harvard Apparatus, Dover, MA, USA) provided the constant flow. The perfusion pressure was measured using a pressure transducer (Pressure Transducer Model P-1000-A, Narco Biosystem, Houston, TX, USA) placed in the circuit between the outlet of the pump and the preparation and was recorded on a Narco physiograph (Desk Model DMP-4B, Narco Biosystem). After 30-min equilibration, each tissue was used for either vasoconstriction or vasorelaxation response as will be described later.

2.4. Vasoconstriction experiment

For measuring the vasoconstriction response of the mesenteric vascular bed, phenylephrine, an α_1-adrenoceptor agonist, was injected (in doses of 0.1 nmol to 1 μmol) into the perfusate before it entered the tissue. The injection volume was 0.1 ml and injection time was 30 s. The vasoconstriction, being recorded as an increase in perfusion pressure, was expressed as mm Hg increase in perfusion pressure.

2.5. Vasorelaxation experiment

After 30-min equilibration, the vascular bed was constricted with Krebs–Henseleit solution containing phenylephrine (0.5 μM for sham-operated and 1 μM for other groups) to induce submaximal vasoconstriction (about 90% of maximum vasoconstriction of the respective groups). Then it was left to reach a plateau and to stabilize for 45 min. Then anandamide was injected (0.1 ml, in 30 s) in doses of 0.1 nmol to 1 μmol (to 10 μmol in anadamide dose–response curve), causing a dose-dependent relaxation, recorded as a decrease in perfusion pressure. The responses were interpreted as percent vasorelaxation of the phenylephrine-induced preconstriction. The perfusate contained

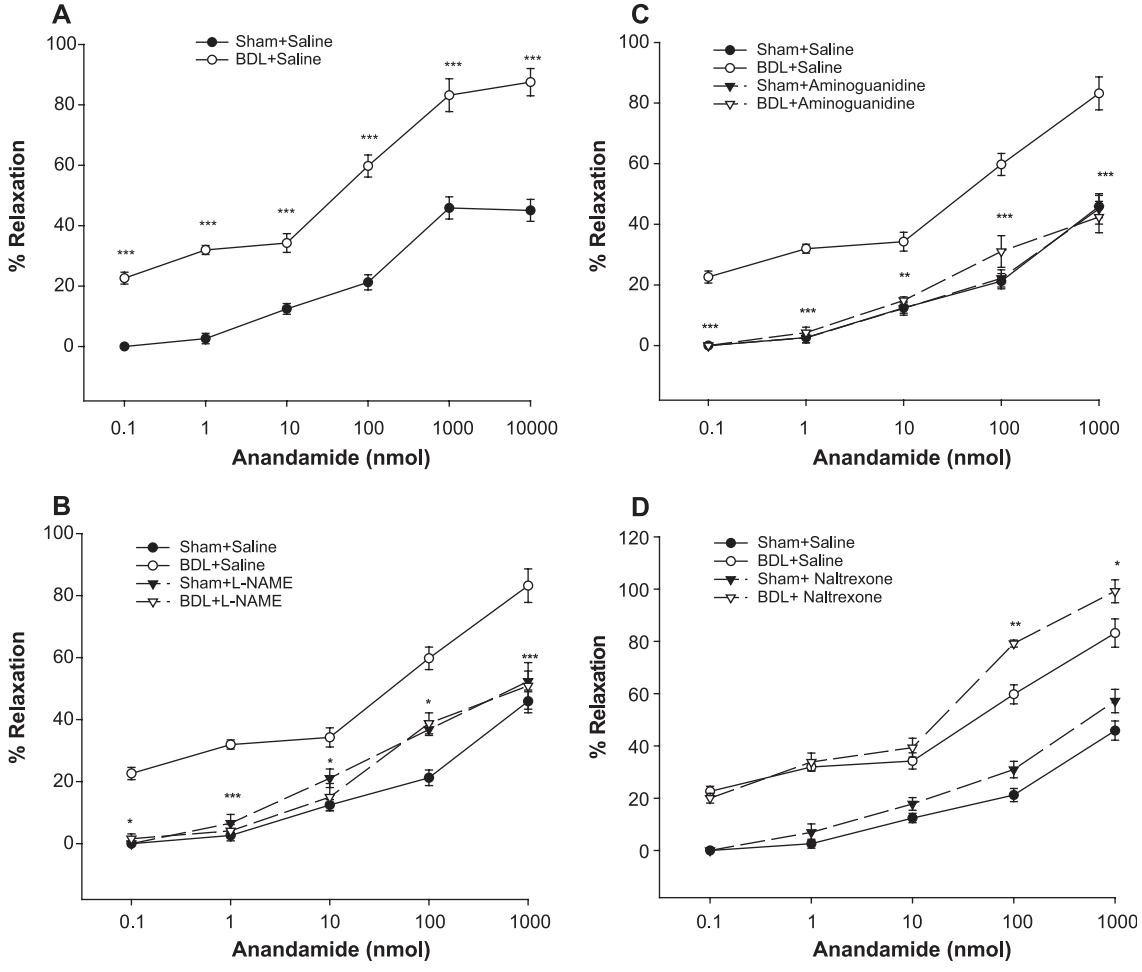

Fig. 2. The vasorelaxation response curve to anandamide for the mesenteric vascular bed, preconstricted with phenylephrine, of (A) sham-operated and bile duct-ligated rats treated with chronic administration of saline; (B) sham-operated and bile duct-ligated rats treated with chronic administration of either saline or L-NAME (20 mg/kg/day, i.p.); (C) sham-operated and bile duct-ligated rats treated with chronic administration of either saline or aminoguanidine (150 mg/kg/day, i.p.); (D) sham-operated and bile duct-ligated rats treated with chronic administration of either saline or naltrexone (10 mg/kg/day, i.p.). Each point represents the mean ± S.E.M. for six to seven rats. $*P<0.05$, $**P<0.01$ and $***P<0.001$ compared with the corresponding sham values in A and BDL-saline values in B, C and D.

100 μM of L-NAME or 1 μM naltrexone in corresponding groups acutely treated with these drugs added 30 min before and during the concentration–response curve for anandamide. The integrity of endothelium was confirmed by the presence of maximal vasorelaxation in response to 1 μmol acetylcholine.

2.6. Drugs

The following drugs were used: phenylephrine hydrochloride, anandamide (*N*-arachidonyl ethanolamide), L-NAME, aminoguanidine and naltrexone (Sigma, St. Louis, MO, USA).

Anandamide was dissolved in 1:1:18 emulphore/ethanol/saline and phenylephrine was dissolved in the perfusate medium, Krebs–Henseleit. Naltrexone, L-NAME and aminoguanidine were dissolved in deionized distilled water for chronic treatment and in the perfusion medium, Krebs–Henseleit containing phenylephrine for acute treatment.

2.7. Statistical analysis

The data are expressed as means ± S.E.M. The two-way analysis of variance followed by Tukey multiple comparisons was used to analyze the data. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Induction of cholestasis

Two days after bile duct ligation, the animals showed signs of cholestasis (jaundice, dark urine and steatorrhea). These signs were confirmed biochemically by a significant rise in the level of serum total bilirubin (5 ± 0.7 μM in sham-operated group versus 95 ± 12.5 μM in bile duct-ligated group) on the seventh day in bile duct-ligated rats ($P < 0.01$). None of the rats showed ascites at the time of the experiment.

3.2. Phenylephrine-induced vasoconstriction

Phenylephrine induced dose-dependent vasoconstriction, manifested as an increase in the perfusion pressure with maximum vasoconstriction achieved at 1 μmol. Seven days after surgery, the bile duct-ligated rats had a significantly lower response to phenylephrine (10 and 100 nmol) ($P < 0.001$), but the maximum response did not differ between the sham-operated and bile duct-ligated rats (Fig. 1). These results are in agreement with our previous results (Namiranian et al., 2001).

3.3. Anandamide-induced vasorelaxation

Anandamide induced a dose-dependent reduction of the contractile responses to phenylephrine in all groups. Anandamide-induced relaxation was significantly potentiated in mesenteric vascular beds of bile duct-ligated rats at all doses ($P < 0.001$) (Fig. 2A).

3.4. Anandamide-induced vasorelaxation during chronic treatment with L-NAME, aminoguanidine or naltrexone

Chronic treatment of bile duct-ligated animals with L-NAME (20 mg/kg/day) or aminoguanidine (150 mg/kg/day) blocked this hyperresponsiveness and the difference in responsiveness was not significant between sham-operated and bile duct-ligated rats after these chronic treatments (Fig. 2B,C).

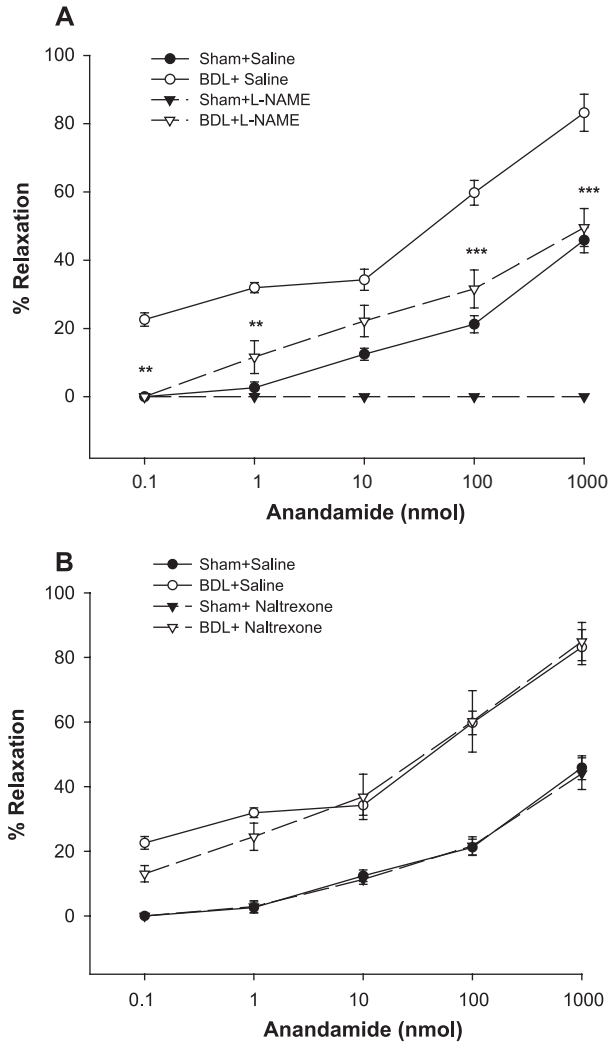

Fig. 3. The vasorelaxation response curve to anandamide for mesenteric vascular bed, preconstricted with phenylephrine, of (A) sham-operated and bile duct-ligated rats treated with saline, or with acute treatment of their mesenteric vascular beds with L-NAME (100 μM); (B) sham-operated and bile duct-ligated rats treated with saline, or with acute treatment of their mesenteric vascular beds with naltrexone (1 μM). Each point represents the mean ± S.E.M. for six to seven rats. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the corresponding BDL-saline values.

Long treatment with naltrexone (10 mg/kg/day for 6 days) did not modify the responses to anandamide in the sham operated rats, but potentiated the responses to 0.1 and 1 μmol anandamide in bile duct-operated rats ($P<0.05$) (Fig. 2D).

3.5. Anandamide-induced vasorelaxation in mesenteric beds acutely treated with L-NAME and naltrexone

Acute L-NAME treatment (100 μM) either completely blocked in the controls, or partially abolished in the bile duct-ligated group, the anandamide-induced relaxations (Fig. 3A). On the contrary, responses to anandamide were unmodified in either group by acute exposure to 1 μM naltrexone (Fig. 3B).

4. Discussion

In the present study, we demonstrated that anandamide-induced relaxations were significantly potentiated in the mesenteric bed of bile duct-ligated rats. The anandamide-induced vasorelaxations were not seen on in vitro exposure of mesenteric vascular beds of sham-operated animals to L-NAME, whereas they were present in the bile duct-ligated rats. Bile duct-ligated rats receiving L-NAME or aminoguanidine for 7 days had restored mesenteric vascular hyperresponsiveness to anandamide in comparison to bile duct-ligated rats treated with saline. Chronic treatment of bile duct-ligated rats with naltrexone somehow increased this hyperresponsiveness. However, this increased vasorelaxation was unmodified by the in vitro exposure of mesenteric vascular beds to naltrexone.

In agreement with results of previous studies the phenylephrine-induced vasoconstriction response of cholestatic rats was impaired (Bomzon et al., 1985; Cioffi et al., 1986; Namiranian et al., 2001). The exact mechanism for this impairment is not yet clearly defined, but the possibility of post-receptor defects, present in many receptors of cirrhotic rats (Jaue et al., 1997), needs to be studied. It is reported that NOS inhibition or endothelium denudation (Utkan et al., 2000; Kimpel et al., 1998) reverses the hyporesponsiveness of vascular elements of cholestatic animals, providing evidence for the role of endothelium-derived NO in this matter.

We showed that bolus doses of anandamide produced dose-dependent vasodilation in mesenteric beds, which is in agreement with previous results (Randall et al., 1996, 1997; Wagner et al., 1999; Mukhopadhyay et al., 2002). The precise mechanism of the vasorelaxation is uncertain, but the vascular action of endocannabinoids suggests that there may be vascular cannabinoid receptors, which may either fall among the classical cannabinoid CB_1 receptors (Onaivi, 2002) or be novel vascular receptors distinct from cannabinoid CB_1 or CB_2 receptors (Offertaler et al., 2003; Jarai et al., 1999; Onaivi, 2002).

It has been shown that changes in the cannabinoid system might be one of the mechanisms responsible for the vasodilated state in chronic liver cirrhosis and a three-fold increase in cannabinoid CB_1 receptors of isolated vascular endothelial cells in cirrhotic human livers has been reported. Low systemic blood pressure of cirrhotic rats is elevated by the cannabinoid CB_1 receptor antagonist, SR141716A and monocytes of these rats have increased levels of anandamide (Batkai et al., 2001).

We have now shown the potentiation of anandamide-induced relaxation in mesenteric beds of bile duct-ligated rats. Several factors may contribute to hyperresponsiveness in these vessels.

Deutsh et al. (1997) have reported that rat cultured renal endothelial cells contain anandamide, together with synthase and amidase activities. Anandamide vasodilator activity of endothelial cells can be terminated by cellular reuptake, which is the rate-limiting step in anandamide degradation, via a specific transporter. It has been shown that NO donors and peroxynitrite cause 2.2- and 4-fold activation of anandamide transport into endothelial cells, respectively (Maccarrone et al., 2000). On the other hand, several studies have reported evidence for NO overproduction (Inan et al., 1997; Nahavandi et al., 1999, 2000; Mani et al., 2001, 2002) in cholestasis. One of the consequences of elevated levels of NO is an increased reaction with superoxide anions (O_2^-) to form peroxynitrite (Squadrito and Pryor, 1998) and there is evidence for in vivo peroxynitrite production in human chronic cirrhosis (Banan et al., 2000). So possibly, an increased level of NO and peroxynitrite in cholestasis can increase the activity of anandamide transporter and thus more endogenous anandamide could be degraded. Therefore, as a result of a compensating mechanism, the upregulation of cannabinoid CB_1 receptors or a novel vascular cannabinoid receptor (Jarai et al., 1999) and increased vasorelaxation in response to anandamide can be seen. This hypothesis is in line with the three-fold increase in cannabinoid CB_1 receptors seen on isolated vascular endothelial cells of cirrhotic rats (Batkai et al., 2001), so it can be suggested that, as in cirrhosis, increased cannabinoid CB_1 receptors may be responsible for hyperresponsiveness in cholestasis.

The supersensitivity of vanilloid VR1 receptors is another possibility. The vasorelaxant effects of anandamide are likely to be at least partially mediated through vanilloid receptors (Zygmunt et al., 1999; Mukhopadhyay et al., 2002). Potentiation of anandamide effects in isolated mesenteric beds from endotoxemic rats 6 h after lipopolysaccharide administration has been reported to be due to supersensitivity of vanilloid receptors during endotoxaemia (Orliac et al., 2003). Therefore, it may be suggested that cholestasis, which is also known to be associated with endotoxaemia (Clements et al., 1998), could cause vanilloid receptor supersensitivity. Further studies using selective agonists and antagonists of CB_1 or VR1 receptors should be done to explore these theories.

In the present study, we have shown that acute L-NAME treatment of mesenteric vascular beds in sham-operated rats completely blocked anandamide-induced vasorelaxation. These data are in accordance with results of other studies that have demonstrated that anandamide, via cannabinoid CB_1 receptors, stimulated renal endothelial cells to release NO (Deutsch et al., 1997) and pretreatment of aortic rings with L-NAME completely blocked methanandamide-induced vasorelaxation (Mukhopadhyay et al., 2002) although other studies have shown different results (Mendizabal et al., 2001; Grainger and Boachie-Ansah, 2001).

Acute L-NAME treatment of mesenteric vascular beds of bile duct-ligated rats could not completely block anandamide-induced vasorelaxation, suggesting a possible involvement of NO overproduction in this response. We also showed that, in chronic L-NAME-treated bile duct-ligated rats, anandamide-induced vasorelaxation was restored toward normal, which might have been due to NO overproduction prevention. These results are in agreement with two possible hypotheses mentioned about upregulation of cannabinoid CB_1 receptor or vanilloid receptor supersensitivity.

Different studies have reported the involvement of iNOS (Knowles et al., 1990; Radomski et al., 1990), endothelial NOS (eNOS) (Weigert et al., 1995) or neuronal NOS (nNOS) (Swain et al., 1997) in different manifestations of cholestasis. In our study, we showed that chronic treatment of cholestatic animals with aminoguanidine, a selective iNOS inhibitor, completely blocked hyperresponsiveness to anandamide, suggesting the role of iNOS in this effect. These results provide further evidence for the role of NO overproduction in complications of cholestasis and suggest a new approach toward managing cholestasis-induced vascular disorders.

Another finding of this study was that the anandamide-induced vasorelaxation was unmodified by the in vitro exposure to naltrexone in both sham-operated and bile duct-ligated groups, whereas it was potentiated in response to large doses of anandamide after in vivo administration of naltrexone as compared to the effect in bile duct-ligated animals treated with saline.

An increased level of endogenous opioid peptides in cholestasis (Dehpour et al., 1998, 1999, 2000; Jones and Bergasa, 2000) may chronically increase intracellular calcium (Way et al., 1998). Anandamide can thus no longer increase the intracellular calcium level to stimulate constructive NOS (cNOS) to release NO. Therefore, blockade of opioid receptors by naltrexone inhibits this increase, and potentiation of hyperresponsiveness to anandamide will be observed.

Another possibility is interaction of cannabinoids and opioids at the level of their signal transduction mechanisms. Receptors for both types are coupled to similar intracellular signaling mechanisms, to a decrease in cAMP production, activation of the potassium or inhibition of calcium channels (Onaivi, 2002) through activation of G_i proteins (Manzanares et al., 1999). It can be speculated that increased level of endogenous opioids in bile duct-ligated rats, using G_i proteins via opioid receptors causes decreased anandamide accessibility to G_i proteins of cannabinoid receptors and consequently potassium and calcium channels. Therefore, it has been supposed that the chronic treatment with naltrexone, occupying opioid receptors, can potentiate the hyperresponsiveness to anandamide.

As a conclusion, there is potentiated anandamide-induced vasorelaxation in the mesenteric vascular beds of bile duct-ligated rats, possibly due to a defect in cannabinoid or vanilloid receptors. NOS inhibition reversed anandamide-induced vascular hyperresponsiveness of bile duct-ligated rats, suggesting a role for increased NO overproduction in this effect.

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