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Endothelin-1 induces vasoconstriction and nitric oxide release via endothelin ET_B receptors in isolated perfused rat liver

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Abstract

Endothelin-1 (0.1, 1 and 10 nM) induced a significant increase in portal pressure and nitric oxide (NO) release in the isolated rat liver. The endothelin ET_B receptor agonist, IRL 1620 (Suc-[Glu^9,$Ala^{11,15}$]endothelin-1-(8–21)) (0.1, 1 and 10 nM) also elicited a marked increase in portal pressure and NO release. The potency of endothelin-1 was higher than that of IRL 1620. The endothelin ET_A receptor antagonist, BQ-123 (*cyclo*(-D-Trp-D-Asp-Pro-D-Val-Leu)) (1 and 10 μM), had no effect on the endothelin-1-induced change in portal pressure and NO current. In contrast, the endothelin ET_B receptor antagonist, BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methyl-leucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine) (1 and 10 nM), attenuated the endothelin-1-induced change in portal pressure and NO current. Administration of N^G-monomethyl-L-arginine (L-NMMA), a NO synthase inhibitor, completely abolished the endothelin-1- or IRL 1620-induced NO release. L-NMMA enhanced the increase in portal pressure and decrease in O_2 consumption caused by endothelin-1. These results indicated that endothelin ET_B receptors mediate both vasoconstriction and NO release and that NO plays a significant role in stabilizing microcirculation in isolated perfused rat liver.

Keywords: Endothelin; Endothelin ET_A receptor; Endothelin ET_B receptor; Pressor effect; Nitric oxide (NO); Oxygen consumption; Liver

1. Introduction

The endothelium participates in the regulation of vascular tone by synthesizing potent vasoactive substances such as endothelin-1, a vasoconstrictor, and nitric oxide (NO), a vasodilator (De Nucci et al., 1988). Endothelin receptors have been classified into two major subtypes, namely endothelin ET_A (which is highly selective for endothelin-1) and ET_B (non-isopeptide selective) (Sakurai et al., 1990). Endothelin ET_A receptors are found predominantly on vascular smooth muscle cells and mediate contraction. In contrast, endothelial cells have endothelin ET_B receptors which are thought to induce relaxation of vascular smooth muscle via nitric oxide production (Namiki et al., 1992; Hirata et al., 1993). The interaction between endothelin and NO is reportedly of importance in the regulation of vascular tone. Endothelin-1 and NO reportedly play a major role in regulating hepatic microcirculation, as with other organs (Zhang et al., 1994; Okumura et al., 1994).

Infusion of endothelin-1 into the intact perfused rat liver causes a profound, sustained increase in hepatic portal pressure and a transient increase in O_2 consumption which is followed by a second phase characterized by a slow decrease (Gandhi et al., 1990; Tran-Thi et al., 1993). As in other organs, two specific endothelin receptors, ET_A and ET_B, have been identified in the liver. Housset et al. (1992) reported that Ito cells (hepatic stellate cells or fat-storing cells) express both endothelin ET_A and ET_B receptors, whereas Kupffer cells and sinusoidal endothelium express only endothelin ET_B receptors. The identity of the endothelin receptor involved in mediating the endothelin-induced intrahepatic vasoconstriction and sinusoidal constriction is still in question. Rockey (1995) reported that the endothelin ET_B receptor is a prominent mediator of Ito cell contraction in vitro and raised the possibility of a novel endothelin receptor subtype. Zhang et al. (1995) demonstrated that the endothelin-1-induced sinusoidal constriction is mediated by endothelin ET_A receptors on Ito cells, and that endothelin-induced intrahepatic vasoconstriction resulted in part from presinusoidal constriction, which was mediated by endothelin ET_B but not ET_A receptors in isolated perfused rat liver.

Direct measurement of endothelin-1-induced NO is required to understand the role of endothelin and NO in the modulation of the intrahepatic microcirculation. However,

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such data are, to our knowledge, unavailable because of the short half-life of NO. Further, there are no studies that demonstrate which receptors mediate the endothelin-induced release of NO in the liver.

The present study was designed to (1) reconfirm the role of endothelin ET_A and ET_B receptors in endothelin-1-induced intrahepatic vasoconstriction in isolated perfused rat liver; (2) investigate which receptor is involved in mediating the endothelin-1-induced NO release; and (3) more closely examine the interaction between endothelin-1 and NO in regulating intrahepatic vasopressure and hepatic O_2 consumption. To this end, we used a relatively specific endothelin ET_A receptor antagonist, BQ-123 (*cyclo*(-D-Trp-D-Asp-Pro-D-Val-Leu)) (Ihara et al., 1992), an endothelin ET_B receptor antagonist, BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine) (Ishikawa et al., 1994), an endothelin ET_B receptor agonist, IRL 1620 (Suc-[Glu^9,$Ala^{11,15}$]endothelin-1-(8–21)) (Takai et al., 1992), and NO-selective electrodes (Ichimori et al., 1994) to continuously monitor the endothelin-1-induced NO release in isolated perfused rat liver.

2. Materials and methods

2.1. Measurement of portal pressure, oxygen consumption and nitric oxide from isolated perfused rat liver

Livers were taken from male Sprague-Dawley rats (310–340 g) under deep sodium pentobarbital (40 mg/kg i.p.) anaesthesia, using a standard technique described earlier (Oshita et al., 1992; Mittal et al., 1994). The perfusate with Krebs-Henseleit bicarbonate buffer (118 mM NaCl, 4.74 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4$, 24.87 mM $NaHCO_3$, and 2.54 mM $CaCl_2$ at pH 7.4, 37°C and saturated with 95% O_2/5% CO_2) was pumped with a rotor pump (Gilson Minipuls III, Middleton, WI, USA) into the liver via a cannula inserted in the portal vein in a haemoglobin-free non-recirculating system at a constant flow rate (40 ml/min), and the effluent escaped through a cannula inserted in the inferior vena cava. The inlet pressure was monitored continuously with a pressure transducer. Needle-type oxygen electrodes (model PO_2-100DW, Intermedical, Tokyo, Japan) were placed in the influent and effluent perfusate to monitor the oxygen partial pressure. Oxygen consumption was calculated from the difference between influent and effluent concentrations, the flow rate, and the liver wet weight.

NO was measured with a commercially available NO meter (model NO-501, Intermedical) which consists of an ammeter with a built-in power supply and an NO-sensitive electrode. The principle behind these measurements and the design of the electrode have been described elsewhere (Ichimori et al., 1994). The electrode was calibrated to NO using *S*-nitroso-*N*-acetyl-DL-penicillamine, a standard NO donor, following methods described previously (Ichimori et al., 1994). With this measuring instrument, a current of 1000 pA is approximately equal to an NO concentration of 1 μM. The working electrode was placed in the effluent perfusate and the counter-electrode was fixed to touch the surface of liver tissue. Baseline measurements were obtained after the basic current had stabilized.

2.2. Study designs

Endothelin-1 or IRL 1620 was introduced into the portal vein cannula for 5 min, at a final concentration of 0.01, 0.1, 1 and 10 nM ($n = 4$–5, respectively), and portal pressure, O_2 consumption and NO output were measured over the next 45 min. In some experiments, an inhibitor of NO synthesis, N^G-monomethyl-L-arginine (L-NMMA) (10 μM in the perfusate), was infused into the livers for 65 min, starting 20 min before the initiation of endothelin-1 or IRL 1620 infusion at each concentration. In other experiments, BQ-123 (1, 10 μM in the perfusate) or BQ-788 (1, 10 nM) was administered into the portal vein cannula for 25 min, starting 20 min before the initiation of endothelin-1 infusion at each concentration. In addition, BQ-123 (10 μM) and BQ-788 (10 nM) were infused simultaneously into the livers before the initiation of endothelin-1 infusion at a final concentration of 0.1, 1 and 10 nM ($n = 4$, respectively).

2.3. Drugs and chemicals

Endothelin-1 and IRL 1620 (Suc-[Glu^9,$Ala^{11,15}$]endothelin-1-(8–21)) were purchased from Peptide Institute (Osaka, Japan) and L-NMMA was purchased from Wako (Osaka, Japan). BQ-123 (*cyclo*(-D-Trp-D-Asp-Pro-D-Val-Leu)) and BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine) were a gift from Banyu Pharmaceuticals (Tsukuba, Japan). All drugs were dissolved in phosphate-buffered saline (pH 7.4) except BQ-788, which was dissolved in 0.01% dimethyl sulfoxide.

2.4. Statistical analysis

The data are presented as means ± S.E.M. Analyses of the data were performed with an analysis of variance (ANOVA) followed by Fisher's post-hoc test when various treatments were compared to the same control groups, or with the Mann-Whitney test for paired or unpaired observations. Differences were considered statistically significant if $P \leq 0.05$.

3. Results

3.1. Portal pressure, oxygen consumption and nitric oxide induced by endothelin-1 and IRL 1620

The baseline liver portal pressure, O_2 consumption and NO current were 4.0 ± 0.0 mmHg, 44.8 ± 0.1 μl/g liver

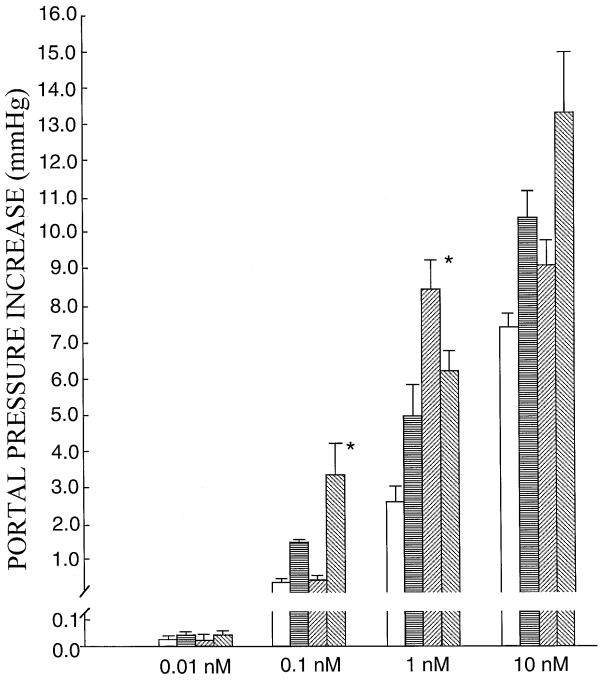

Fig. 1. Dose-dependent pressor effects of IRL 1620 (0.01, 0.1, 1 and 10 nM; open bars) and endothelin-1 (0.01, 0.1, 1 and 10 nM; horizontally striped bars) in the absence or in the presence of L-NMMA (IRL 1620, right-hatched bars; endothelin-1, left-hatched bars). The increase in portal pressure was the difference between the peak value and the basal one. * $P \leq 0.05$ when compared with the values obtained without added L-NMMA.

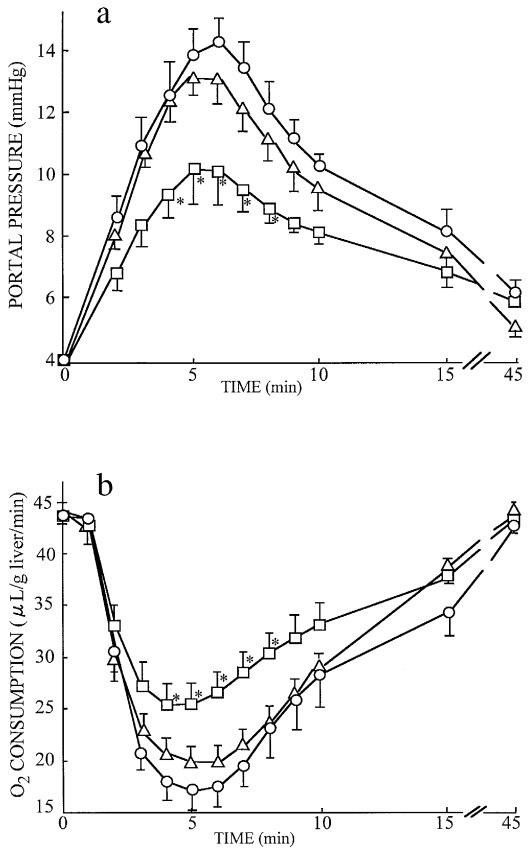

Fig. 3. Time-course of portal pressure (a) and O_2 consumption (b) in isolated perfused rat liver with endothelin-1 (10 nM) in the absence (○) or presence of BQ-123 at 10 μM (△), and BQ-788 at 10 nM (□). Endothelin-1 was infused at time 0 for 5 min. BQ-123 and BQ-788 were added at time −20 for 25 min. Values represent the means ± S.E.M. for 5 experiments. * $P \leq 0.05$ when compared with the values without added antagonist at each time point.

per min and 493.8 ± 4.0 nmol, respectively ($n = 149$). Infusion of endothelin-1 (0.01 nM) did not significantly change the portal pressure, O_2 consumption, or nitric oxide current (Figs. 1 and 2). Portal pressure increased immediately after the addition of endothelin-1 (1 and 10 nM) and reached a maximum in approximately 5.5 min, then gradually decreased. O_2 consumption decreased after the infusion of endothelin-1; thereafter it recovered slowly over the next 20 min (Fig. 3). At high doses of endothelin-1 (10 nM), hepatic O_2 consumption declined significantly to

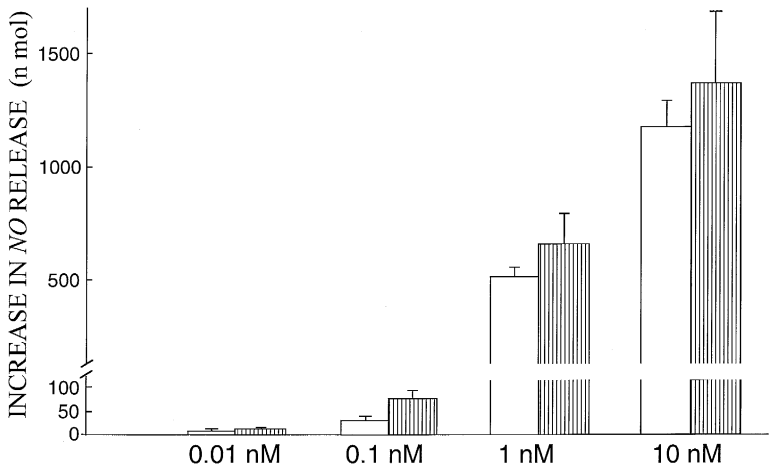

Fig. 2. Dose-dependent effects on NO release with IRL 1620 (0.01, 0.1, 1 and 10 nM; open bars) and endothelin-1 (0.01, 0.1, 1 and 10 nM; vertical striped bars) ($n = 4–5$, respectively). The increase in NO current was the difference between the peak value and the basal one.

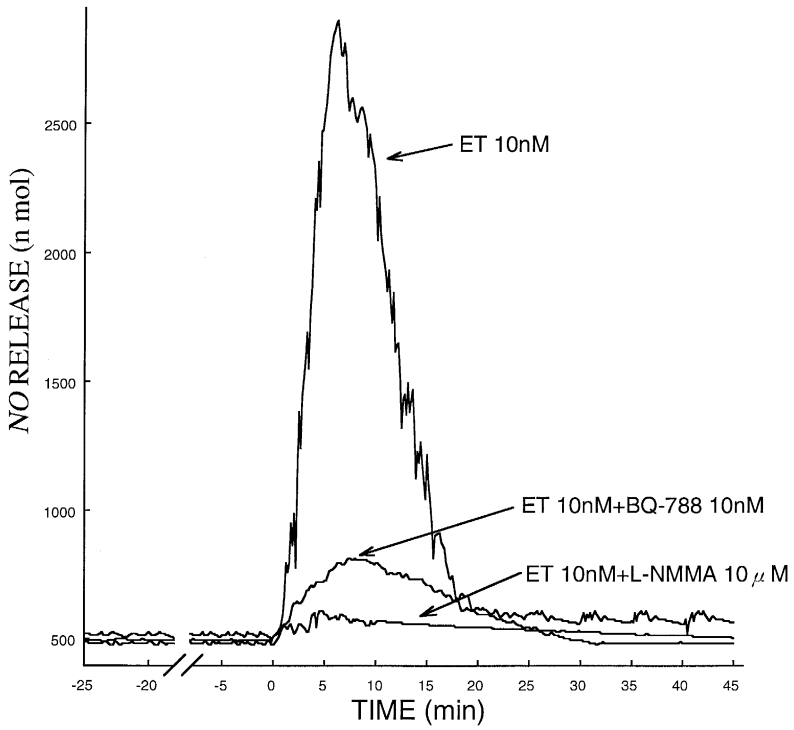

Fig. 4. Representative tracing of nitric oxide (NO) current in the isolated perfused rat liver during the infusion of endothelin-1 (ET), endothelin ET_B receptor antagonist, BQ-788 or N^G-monomethyl-L-arginine (L-NMMA). Endothelin-1 (10 nM) was infused at time 0 for 5 min. BQ-788 (10 nM) was infused at time −20 for 25 min. L-NMMA (10 μM) was added at time −20 for 65 min.

about 40% of the basal level. The NO current increased following infusion of endothelin-1 (Figs. 3–5). NO current reached a maximum 6 min after the addition of endothelin-1, and NO release continued for at least 25 min more. Infusion of endothelin-1 at 0.1 nM caused a similar change in portal pressure and NO current. However, O_2 consumption increased slightly after the addition of endothelin-1, then recovered slowly over the next 20 min (data not shown).

IRL 1620 elicited changes in portal pressure, O_2 consumption and NO current similar to those with endothelin-1, although it was less potent (Figs. 1 and 2). Infusion of IRL 1620 (0.01 nM) produced no significant change in portal pressure and NO current, but the values increased

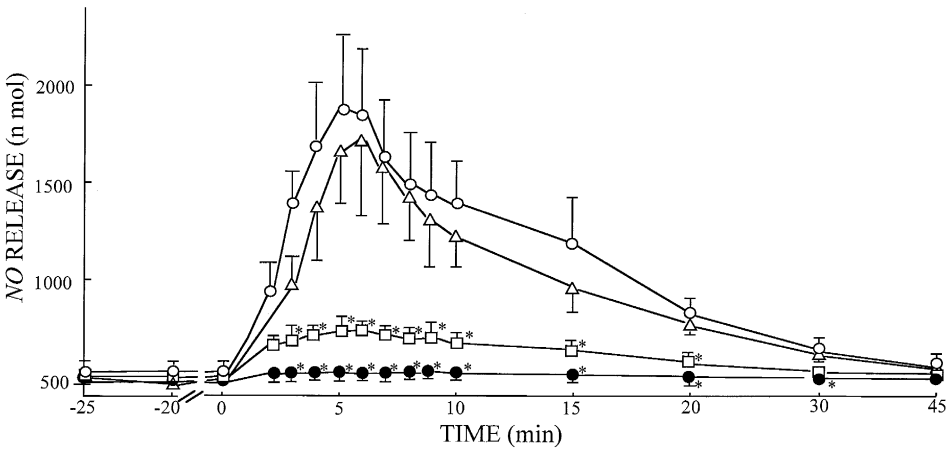

Fig. 5. Time-course of nitric oxide (NO) current induced by endothelin-1 in isolated perfused rat liver (10 nM) in the absence (○) or presence of BQ-123 at 10 μM (△), BQ-788 at 10 nM (□) and L-NMMA (10 μM; ●). Endothelin-1 was infused at time 0 for 5 min. BQ-123 and BQ-788 were added at time −20 for 25 min. L-NMMA (10 μM) was added at time −20 for 65 min. Values represent the means ± S.E.M. for 5 experiments. * $P \leq 0.05$ when compared with the values obtained without added antagonist at each time point.

after the infusion of higher concentrations (0.1, 1 and 10 nM) of IRL 1620. At 10 nM of IRL 1620, the mean O_2 consumption decreased to about 50% of the basal value (22.8 μl/g liver per min).

3.2. Effects of BQ-123 and BQ-788 on endothelin-1-induced changes in portal pressure, oxygen consumption and nitric oxide release

Infusion of either BQ-123 or BQ-788 for 20 min before the addition of endothelin-1 had no effect on the baseline liver portal pressure, O_2 consumption and NO current. BQ-123 at 1 and 10 μM did not affect the endothelin-1-induced change in portal pressure, O_2 consumption and NO current. In contrast, BQ-788 (1 and 10 nM) significantly attenuated the change in portal pressure, O_2 consumption and NO current induced by endothelin-1 (Figs. 3–5). When BQ-123 (10 μM) and BQ-788 (10 nM) were administered simultaneously, the results were the same as when BQ-788 was given alone (data not shown).

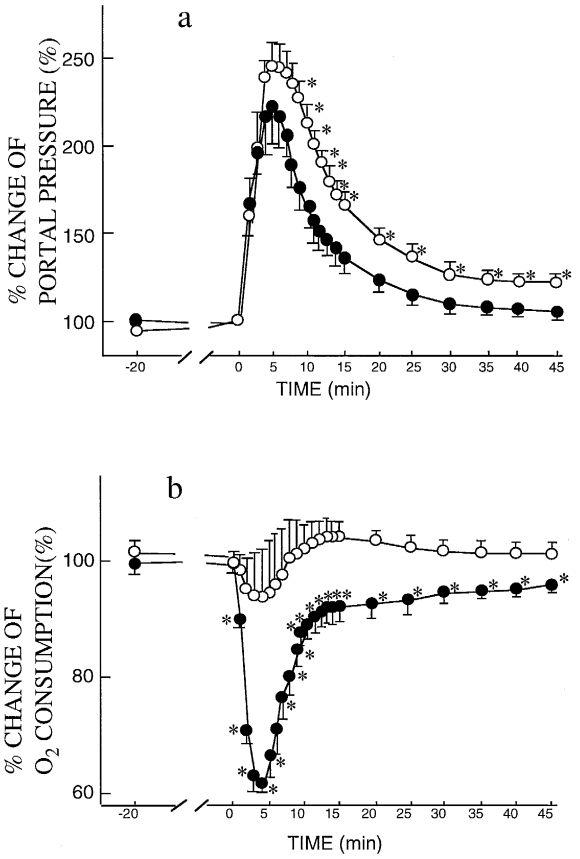

Fig. 6. Time-course of portal pressure (a) and O_2 consumption (b) induced by endothelin-1 (1 nM) in isolated perfused rat liver (1 nM) in the absence (○) or presence of L-NMMA (10 μM, ●). Endothelin-1 was infused at time 0 for 5 min. L-NMMA (10 μM) was added at time −20 for 65 min. Data are given as percentages of the respective baseline value at time 0. Each value represents the mean ± S.E.M. for 5 experiments. * $P \leq 0.05$ when compared with the values obtained without added antagonist at each time point.

3.3. Effects of L-NMMA on endothelin-1- and IRL 1620-induced changes in portal pressure and oxygen consumption

L-NMMA infusion completely inhibited the NO response to endothelin-1 (Figs. 4 and 5). Infusion of L-NMMA for 20 min slightly but significantly increased both portal pressure (4.0 ± 0.0 vs. 4.2 ± 0.0, $n = 38$, $P < 0.001$) and O_2 consumption (44.6 ± 0.2 vs. 45.7 ± 0.3, $n = 38$, $P < 0.001$). Therefore, we compared the change in portal pressure and O_2 consumption in the presence and absence of L-NMMA, using the percent change from the value obtained before the infusion of endothelin-1. In livers pre-infused with L-NMMA for 20 min prior to endothelin-1 (1 nM) infusion, the endothelin-1 infusion increased portal pressure and decreased O_2 consumption significantly more than in the absence of L-NMMA (Fig. 6). Pre-infusion with L-NMMA prior to endothelin-1 (0.1 and 10 nM) also significantly enhanced the change in portal pressure and O_2 consumption. Simultaneous infusion of L-NMMA with IRL 1620 also significantly augmented the change in portal pressure and O_2 consumption (data not shown).

4. Discussion

The use of NO-selective electrodes in the current study allowed us to obtain simultaneous real-time measurements of NO release, hepatic portal pressure and hepatic O_2 consumption. L-NMMA infusion completely inhibited the NO response to endothelin-1. However, in the absence of L-NMMA, endogenous NO was released immediately after endothelin-1 induction; release reached a maximum after the increase in portal pressure peaked and thereafter gradually returned to its basal level. Therefore, it appears that endothelin-1 released endogenous NO, which then acted as a vasodilator. It is suggested that NO plays a significant role in the stabilization of the hepatic microcirculation, thereby helping to protect against hepatocellular damage from endothelin-1-induced vasoconstriction. Further, the present study demonstrates that endothelin ET_B receptors mediate vasoconstriction and NO release, because an endothelin ET_B receptor antagonist, BQ-788, inhibited hepatic vasoconstriction and nitric oxide release. In addition, an endothelin ET_B receptor agonist, IRL 1620, elicited an increase in hepatic portal pressure and NO release.

Binding experiments performed by Housset et al. (1992) indicated that the number of endothelin receptors per cell is highest on Ito cells. The authors also reported that both endothelin ET_A and ET_B receptors were expressed by Ito cells, that only endothelin ET_B mRNA was detectable in sinusoidal endothelial cells and in Kupffer cells and that the level of receptor mRNA was markedly greater in Ito cells than in the other cell types. Light and electron-microscopic autoradiography revealed that endothelin-1 binds to

Ito cells in Disse's spaces (Gondo et al., 1993). Moreover, there is accumulating evidence that Ito cells have contractile properties (Kawada et al., 1993; Housset et al., 1992). Therefore, it has been suggested that Ito cells are a major target for endothelin-1 in the liver (Kawada et al., 1993; Housset et al., 1992, 1995).

Preinfusion of BQ-123 did not affect endothelin-1-induced intrahepatic vasoconstriction. This result supports the concept of Rockey (1995), who proposed that the endothelin ET_B receptor is a prominent mediator of Ito cells. The lack of an inhibitory effect of BQ-123 on endothelin-1-induced intrahepatic vasoconstriction is in agreement with the report by Zhang et al. (1995). However, the latter reported that the sinusoidal constriction induced by endothelin-1 was completely abolished by pretreatment with BQ-123, regardless of a lack of effect of BQ-123 on the endothelin-1-induced increase in portal haemodynamics. Although high doses of endothelin-1 also cause contraction of presinusoidal portions of the portal vein in addition to sinusoid, low doses of endothelin-1 act mainly at the sinusoid level (Zhang et al., 1994). In the present study, there was no significant effect of BQ-123 on the increase in portal pressure induced by a low dose of endothelin-1 (0.1 nM); this finding was assumed to reflect the lower probability of presinusoidal constriction. Consequently, parameters measured extrahepatically, such as portal pressure changes, might be insensitive to a physiologically important redistribution of microvascular flow. Therefore, we cannot conclude that the endothelin ET_A receptor was not involved in the mediation of endothelin-1-induced vasoconstriction in this study.

It appeared that the endothelin ET_B receptor mediated vasoconstriction, because, in contrast to that with BQ-123, pretreatment with BQ-788 (1 and 10 nM), significantly inhibited the increase in hepatic portal pressure induced by endothelin-1 infusion. This conclusion was further supported by the fact that the endothelin ET_B receptor agonist, IRL 1620, elicited an increase in portal pressure. However, the inhibition by BQ-788 of the endothelin-1-induced increase in portal pressure was incomplete. In addition, the inhibition by the combination of BQ-123 and BQ-788 was also incomplete. These results could indicate either that the antagonists were not effective or the presence of another receptor type. The incomplete block by BQ-788 alone was also consistent with results of the study by Zhang et al. (1995) who used the endothelin ET_B receptor antagonist, IRL 1038 (1 μM); these authors speculated that the incomplete block of the endothelin ET_B receptors was a result of the unreliability of IRL 1038 (Urade et al., 1994). In the current study, we used a potent and selective endothelin ET_B receptor antagonist, BQ-788. This compound patently and competitively inhibits ^{125}I-labeled endothelin-1 binding to endothelin ET_B receptors on human Girardi heart cells ($IC_{50} = 1.2$ nM) (Ishikawa et al., 1994). Therefore, the possibility of incomplete blocking of the endothelin ET_B receptors is unlikely but cannot be completely excluded. It should be taken into account that the effect of BQ-788 on perfusion portal pressure is complex, because BQ-788 inhibited NO release in addition to vasoconstriction. It might be possible that the inhibition of the increase in portal pressure caused by BQ-788 was attenuated because of the suppression of the vasodilator effect.

The selective endothelin ET_A receptor antagonist, BQ-123, did not affect the increase in NO current caused by endothelin-1. On the other hand, the endothelin ET_B receptor antagonist, BQ-788, reduced NO release. Furthermore, the endothelin ET_B receptor agonist, IRL 1620, elicited an increase in the NO current. Our data could indicate that the endothelin-1-induced NO release is mediated by endothelin ET_B receptors. The origin of NO was not determined in the present study. Furthermore, in preliminary experiments, we observed that NO release occurred after the increase in portal pressure induced by noradrenaline, which promotes vasoconstriction via adrenoceptors. These results indicate that NO release may occur in response to activation of other receptors besides endothelin receptors. However, since NO is a paracrine mediator and modifies the effect of endothelin-1, the NO-secreting cell must be located at or near to the main endothelin site of action (Mittal et al., 1994). It has been assumed that NO facilitates the relaxation of Ito cells. Ito cells are perisinusoidal cells which may be related to tissue pericytes and have been implicated in the regulation of sinusoidal blood flow (Kawada et al., 1993). Therefore, Ito cells might be candidates for NO-secreting cells.

In an attempt to evaluate the effect of NO release induced by endothelin-1 or IRL 1620 on the O_2 supply to hepatic tissue, we measured the O_2 consumption of hepatic cells. Although portal pressure changed in a dose-dependent manner, the change in hepatic O_2 consumption was biphasic. These data suggested that the slight vasoconstriction required an O_2 supply rather than impaired the O_2 supply to hepatic tissue. When NO release was inhibited by L-NMMA, the decrease in hepatic O_2 consumption caused by endothelin-1 (1 and 10 nM) or IRL 1620 (10 nM) was significantly augmented. Compared with the effect of 1 nM of BQ-788, 10 nM of BQ-788, which abolished the endothelin-1-induced NO release, enhanced the decrease in hepatic O_2 consumption caused by endothelin-1 (10 nM). These data indicate that NO acted as a vasodilator and thereby improved the O_2 supply to hepatic tissue by reducing the endothelin-1-induced hepatic vascular contraction, with a consequent increase in hepatic O_2 consumption. Although we did not evaluate hepatocellular damage in this study, it has been reported that hepatic vasoconstriction impairs O_2 supply to the tissue, leading to hepatic tissue hypoxia and reduction of mitochondrial cytochromes and, eventually, cell death (Hijioka et al., 1991; Oshita et al., 1992; Okumura et al., 1994). Therefore, it is suggested that in the presence of L-NMMA, a sustained decrease in hepatic tissue oxygenation may result in hepatocellular damage.

Infusion of L-NMMA somewhat affected the values of both portal pressure and O_2 consumption. The slight change in portal pressure induced by L-NMMA (10 μM) was consistent with findings of Mittal et al. (1994) who used N^G-nitro-L-arginine (L-NAME) (10 μM), an inhibitor of NO synthesis. Suematsu et al. (1995) also reported that, in isolated liver, zinc protoporphyrin-IX (1 and 10 μM), a heme oxygenase inhibitor, induced a significant increase in portal pressure, whereas higher concentrations (100 μM and 1 mM) of L-NAME did not. Their results suggest that under the experimental conditions used in those studies, endogenous NO plays only a minor role in the modulation of the hepatic microcirculation, chiefly at low levels of portal pressure. Under normal conditions, endogenous carbon monoxide rather than NO may contribute to the maintenance of low portal pressure (Suematsu et al., 1995). However, the present study demonstrated that, under more abnormal conditions, vasoconstriction is involved, with a consequent release of NO, which then plays a major role in stabilizing the hepatic microcirculation. In addition to NO, endothelin-1 reportedly releases prostacyclin, which acts as a vasodilator substance in isolated guinea pig or rat lung and bovine aortic endothelial cells (De Nucci et al., 1988; Filep et al., 1991). Endothelin-3 has recently been reported to induce prostaglandin E_2 production in Kupffer cells (Gandhi et al., 1990), whereas endothelin-1 does not promote prostaglandin production in these cells (Kawada et al., 1993). Moreover, preincubation of the preparation with indomethacin did not alter the response to endothelin-1 or norepinephrine in isolated perfused rat liver (Tran-Thi et al., 1993; Mittal et al., 1994). Consequently prostacyclin does not play a significant role in modulating the response to vasoconstriction in the liver. However, further experiments are needed to determine whether or not endothelin-1 stimulates the formation of endogenous prostacyclin.

In summary, the use of NO-selective electrodes allowed us to directly observe the NO release after stimulation of endothelin ET_B receptors, and to find that the released NO counteracted the endothelin-1-induced vasoconstriction.

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