British Journal of Pharmacology (2001) 132, 309–317

www.nature.com/bjp

Gastric mucosal resistance to acute injury in experimental portal hypertension

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1 The gastric mucosa of portal hypertensive rats exhibits important microvascular changes and a nitric oxide (NO)-dependent hyperemia. This study analyses whether portal hypertensive mucosa exhibits changes in its ability to withstand aggression.

2 Portal hypertension was induced by partial portal vein ligation (PPVL) or common bile duct ligation (CBDL) and gastric damage was induced by oral administration of ethanol or aspirin. Experiments were performed in conscious or anaesthetized rats and some animals were pre-treated with the NO-synthesis inhibitor L-NAME.

3 Conscious PPVL or CBDL rats showed an increased resistance to the damaging effects of ethanol. Oral administration of aspirin produced less gastric damage in PPVL conscious rats than in the control group.

4 The protective effects of portal hypertension were maintained in animals anaesthetized with ketamine and absent when pentobarbital was employed.

5 Pre-treatment with L-NAME restored the damaging effects of ethanol and aspirin in PPVL rats without modifying the level of damage in control animals.

6 Gastric bleeding induced by oral aspirin, as measured by the luminal release of ^{51}Cr-labelled erythrocytes, was significantly greater in PPVL rats than in control animals.

7 Semi-quantitiative analysis by RT–PCR of the mRNA for endothelial NO-synthase (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) levels showed that the expression of iNOS was slightly increased in both the gastric mucosa and smooth muscle of PPVL rats. No changes were observed in eNOS and nNOS expression.

8 Conscious portal hypertensive rats exhibit an enhanced resistance to acute gastric damage which is absent under the influence of some types of anaesthesia and seems related to an increased synthesis of nitric oxide. However, mucosal lesions in these animals show an augmented bleeding per area of injury.

British Journal of Pharmacology (2001) **132,** 309–317

Keywords: Portal hypertension; cirrhosis; partial portal vein ligation; common bile duct ligation; gastric damage; gastric bleeding; ethanol; aspirin; nitric oxide; nitric oxide synthase

Abbreviations: CBDL, common bile duct ligation; eNOS, endothelial NO-synthase; iNOS, inducible NO-synthase; L-NAME, N^G-nitro-L-arginine methyl ester; nNOS, neuronal NO-synthase; NO, nitric oxide; NOS, NO-synthase; PPVL, partial portal vein ligation; SO, sham operation

Introduction

Portal hypertensive gastropathy, macroscopically characterized by a pink mosaic pattern and diffused red mucosal spots, is a common feature of the cirrhotic stomach and may also be observed in patients with pre-hepatic portal hypertension (Piqué, 1997). Morphological studies have demonstrated that the histological lesions characteristic of this condition consist of enlarged mucosal and submucosal vessels with little or no inflammatory infiltrate or epithelial erosions (McCormack *et al*., 1985; Quintero *et al*., 1987). Dilation of the gastric mucosal vessels is also a feature of several experimental models of this disease (Albillos *et al*., 1992; Panés *et al*., 1994; Tarnawski *et al*., 1988), and functional studies have demonstrated the existence of important changes in vascular reactivity (Moreno *et al*., 1996; Martinez-Cuesta *et al*., 1996). Furthermore, the rupture of such vessels is responsible for the diffused gastric bleeding that frequently accompanies severe forms of portal hypertensive gastropathy (D'Amico *et al*., 1990; Groszmann & Colombato, 1988; Gupta *et al*., 1997).

It has been proposed that the production of nitric oxide (NO) is increased in the gastric mucosa of portal hypertensive animals and that such augmented levels of NO play a major role in the splanchnic hyperdynamic circulation which characterizes this disease (Casadevall *et al*., 1993; Pizcueta *et al*., 1992). NO is generally considered to exert gastroprotective effects (Whittle, 1994), but several experimental studies have suggested that the mucosa of portal hypertensive animals is less resistant to acute gastric injury. However,

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most of these studies were carried out in an acute model of portal hypertension involving a two-step total portal vein ligation (Sarfeh *et al*., 1983; 1988) or in anaesthetized animals (Back *et al*., 1992; Ferraz *et al*., 1997). Animals undergoing a total portal vein ligation suffer noticeable portal, splacnic and mesenteric vein congestion as a consequence of the complete cessation of the portal vein flow, something that does not occur in cirrhotic patients. Alternatively, we have evaluated the damaging effects of various injurious agents on the gastric mucosa of rats with partial ligation of the portal vein (PPVL). This is a model of pure chronic portal hypertension that reproduces the hemodynamic alterations appearing in clinical conditions (Bosch *et al*., 1992; Gupta *et al*., 1997). To characterize the model-specificity of the results we have also employed rats with biliary cirrhosis induced by common bile duct ligation (CBDL). In this latter model, portal hypertension is not due to surgical interference with the flow of the portal vein, but rather hepatic damage. In addition, the influence that anaesthesia might exert on the susceptibility to damage in these animals has also been considered. Finally, we have evaluated whether there is an enhanced production of NO in PPVL animals which modulates the ability of the mucosa to defend itself against injury and the possible source of such NO.

Methods

Animal preparation

Portal hypertension was induced in male Sprague-Dawley rats (250–300 g body weight) anaesthetized with ketamine (100 mg kg^{-1}, i.m.) using two procedures. Prehepatic portal hypertension was provoked by partial portal vein ligation (PPVL) as previously described (Chojkier & Groszmann, 1981). In brief, the portal vein was isolated and a stenosis created by placing a single ligature of 3-0 silk around both the portal vein and a 20-gauge blunt-tipped needle. The needle was then removed from the ligature, creating a calibrated constriction of the portal vein. In another experimental group, biliary cirrhosis with intrahepatic portal hypertension was induced by ligation and section of the common bile duct (CBDL) (Lee *et al*., 1986). These animals were treated with vitamin K (8 mg kg^{-1}), administered by intramuscular injection 1 week prior to the experiments. In sham-operated (SO) rats, the portal vein or common bile duct were isolated but not ligated. Studies were performed in 24 h fasted rats 14 days after PPVL or 28 days after CBDL. The development of portal hypertension in each animal was confirmed by measuring portal pressure (mmHg) at the end of the experiment. The presence of cirrhosis in CBDL animals was assessed by the presence of ascites or, in non-ascitic rats, by histological analysis of the liver. Blood samples were taken from some animals and mixed with tri-sodium citrate (0.129 M) for automatic evaluation of thrombin time, activated partial thromboplastin time and prothrombin time. All animals were treated according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences. Anaesthesia was assessed in all cases by the absence of withdrawal reflexes, and additional doses of anaesthetic were administered when required.

Macroscopic gastric damage studies

Experiments in conscious animals Ethanol 50% (1 ml rat^{-1}) and aspirin (200 mg kg^{-1} in 0.2 M HCl, 4 ml kg^{-1}) were administered orally and allowed to act on the gastric mucosa for 10 min and 3 h respectively. Saline was applied in control experiments to rule out the possibility of mechanical damage by the oral intubation. Some animals were pre-treated with N^G-nitro-L-arginine methyl ester (L-NAME, 3 mg kg^{-1} i.p., −30 min) or indomethacin (2 or 5 mg kg^{-1} s.c., −60 min).

In a series of experiments, gastric emptying of liquids was evaluated by adding the non-absorbent marker phenol red to the aspirin suspension. We measured the gastric emptying of 1.5 ml of a suspension containing methylcelulosa (1.5%), aspirin (50 mg ml^{-1}) and phenol red (50 mg ml^{-1}). This solution was dispersed in 0.2 M HCl and administered through an orogastric cannula. Animals were killed by CO_2 inhalation 3 h later, except for those in a control group which were killed immediately after administration of the test meal. In all cases, the stomach was clamped at the pylorus and cardia, removed and homogenized in 100 ml of 0.1 N NaOH. The colorimetric assay of phenol red was performed at 560 nm after protein precipitation (20% trichloroacetic acid) and re-alkalinization of the supernatant (NaOH 0.5 N). Gastric emptying was calculated from the formula: $\%GE = [1 - (\text{absorbance}/100\% \text{ absorbance})] \cdot 100$, where absorbance is the value at 560 nm of each sample and 100% absorbance is the average absorbance of samples at this wavelength recovered from rats sacrificed immediately after meal administration.

Experiments in anaesthetised animals An *ex vivo* gastric chamber was prepared in pentobarbital (50 mg kg^{-1}, i.p.) anaesthetised rats as described previously (Beck *et al*., 1992). A midline incision was performed and the pylorus was ligated with 4-0 silk. Afterwards, the stomach was opened along the greater curvature, pinned over a plexiglas platform, and clamped with a plexiglas cylinder to form a gastric chamber. All surgery was performed using a cautery unit to minimise blood loss. The gastric mucosa was bathed for 20 min. in a continuously renewed solution of 200 mmol l^{-1} mannitol and 0.05 M HCl and, thereafter, this liquid was exchanged for 5 ml of ethanol 100%. Ten minutes later, ethanol was removed and perfusion with the previous solution was re-initiated for an additional period of 20 min. Absolute ethanol was used in these experiments in order to obtain a control response (area of gastric damage) similar to that induced by ethanol 50% in conscious animals.

In a further group of animals, 100% ethanol (1 ml rat^{-1}) was administered via an orogastric tube to intact animals anaesthetised with either pentobarbital (50 mg kg^{-1}, i.p.) or ketamine (100 mg kg^{-1}, i.m.), and left in contact with the gastric mucosa for ten minutes.

Evaluation of gastric damage Animals were sacrificed by cervical dislocation. The stomach was removed, opened along the greater curvature, pinned to a wax block, coded to avoid observer bias and photographed on colour transparency film. The area of macroscopically visible damage was calculated *via* computerized planimetry and expressed as the percentage (%) of the total gastric mucosa showing injury (Barrachina *et al*., 1995). All measurements were taken by an observer who was unaware of the treatments previously administered.

Gastric bleeding studies Bleeding from gastric lesions in the mucosa was evaluated by measuring the appearance of ^{51}Cr-labelled erythrocytes in the gastric lumen. Rat blood was collected *via* the carotid artery cannulation into tri-sodium citrate (0.129 M) and then centrifuged ($250 \times g$ for 20 min). Erythrocytes (150 μl volume per recipient rat) were collected from the bottom of the resulting pellet and incubated (30 min, room temperature) with $Na_2{}^{51}CrO_4$ (80 μl, 80 mCi). Tyrode solution was subsequently added to increase the total volume 10 fold and the cell suspensions were centrifuged ($250 \times g$ for 10 min). Erythrocytes were washed in this way three times, and finally re-suspended in a volume of 500 μl per recipient rat (150–200 mCi kg^{-1}). ^{51}Cr-labelled erythrocytes (500 μl) were injected into the tail vein under ether anaesthesia 1 h before aspirin (200 mg kg^{-1} in 0.2 N HCl, 4 ml kg^{-1}, p.o.) administration. Three hours after aspirin intake, rats were anaesthetized with urethane (1.25 mg kg^{-1}, i.p. 10 ml kg^{-1}) and 5 ml of blood were withdrawn from the carotid artery. Two ligatures were placed in the abdominal oesophagus and the proximal duodenum and the stomach was then removed. Macroscopic gastric damage was evaluated. Radioactivity in luminal contents, blood and plasma samples was counted with a gamma counter. Accumulation of ^{51}Cr-labelled erythrocytes in the lumen was standardized by dividing the ^{51}Cr-counts present in the lumen sample by that present in 1 μl whole blood and results were expressed as equivalent μl of blood.

Statistical analysis All data are expressed as mean $\pm$ s.e.mean. Comparisons between three or more groups were made by an analysis of variance (ANOVA), followed by a Student-Newman-Keuls test. Comparisons between two groups were performed by Student's *t*-test. *P* values of less than 0.05 were considered significant.

Materials Aspirin and phenol red (Sigma Chemical Co.) were suspended in HCl 0.2 N with metilcellulose (1.5% p/v). L-NAME (Sigma Chemical Co.), urethane (Sigma Chemical Co.) and $^{51}Na_2CrO_4$ (Amersham) were dissolved in saline. Indomethacin (Sigma Chemical Co.) was dissolved in 5% of sodium bicarbonate. Vitamin K (Fitomenadione®, Konakion Roche), sodium pentobarbitone (Pentothal®, Abbot) and ketamine (Ketolar®, Parke-Davis) were used as clinically available preparations. Unless stated otherwise, all drugs were administered in a volume of 1 ml kg^{-1}.

Gene expression of NOS isoforms

RNA isolation and cDNA synthesis Gastric specimens for sham and PPVL rats were immediately frozen in liquid nitrogen. Gastric mucosa was scraped off and separated from the smooth muscle and frozen specimens were homogenated with Tripure Isolation Reagent (Roche Diagnostics) to isolate total RNA. The RNA concentration in each sample was determined spectrophotometrically, and the quality of each RNA preparation was documented by visualization of 18S and 28S ribosomal bands after electrophoresis through a 1% agarose gel (Hispanlab) and ethidium bromide staining (Serva).

For cDNA synthesis, 3 μg of total RNA and 0.8 μg of oligo(dT) 16 primer (Roche Diagnostics) were preheated at 70°C in diethyl-pyrocarbonate (DEPC)-treated water, and cooled on ice. Reactions (20 μl) contained (mM) Tris-HCl (pH 8.3) 50, KCl 75, $MgCl_2$ 3, dithiothreitol (DTT) 10, 40 units of RNase inhibitor (Roche Diagnostics), 500 μM of each dNTP and 300 units of Superscript Reverse Transcriptase (Life Technologies). The reactions were incubated at 42°C for 1 h.

Semi-quantitative RT-PCR analysis PCR was performed on the aliquots in the resulting cDNA with the use of specific oligonucleotide primers for each isoform (Roche Diagnostics). The specific primer set used for rat eNOS, nNOS, iNOS and cyclophilin is shown in Table 1. PCR reactions (25 μl) were prepared with 10 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, 50 mM KCl, 200 μM of each dNTP, 5 pmol of each primer and 1 U Taq DNA polymerase (Roche Diagnostics), using as a template 3 μl of the above reverse transcription product for amplified iNOS and nNOS fragments. A PCR reaction of 50 μl was performed to amplify eNOS. Parallel reactions were carried out with an internal control of cyclophilin obtained from 1 μl of RT reaction. After an initial 2 min denaturing step at 94°C, between 28 and 42 cycles were carried out for each fragment, the number of cycles and the annealing temperature depending on the set of primers used (see Table 1). An extension step was carried out at 72°C lasting 20 s for nNOS and iNOS and 1 min for eNOS. Samples from the PCR reactions (7.5 μl of nNOS and iNOS and 20 μl of eNOS) were taken at the end of the various cycles and analysed by electrophoresis in 1.5% agarose gel stained with ethidium bromide.

Results

PPVL rats showed a significant increase in portal pressure when compared to sham-operated animals (11.4 $\pm$ 0.7 vs 7.2 $\pm$ 0.7 mmHg respectively, $P<0.001$). Likewise, in CBDL rats, portal pressure was significantly higher than that observed in the control group (13.0 $\pm$ 1.4 vs 7.1 $\pm$ 0.5 mmHg, $P<0.001$). The level of portal hypertension was not affected by the different treatments administered.

Macroscopic gastric damage studies

Conscious animals As shown in Figure 1, the administration of 1 ml of 50% ethanol (p.o.) to conscious rats with portal hypertension induced by PPVL resulted in an area of injury that was 81 $\pm$ 5% smaller ($P<0.05$) than that observed in sham-operated animals. Likewise, CBDL rats exhibited a significantly lower level of damage (72 $\pm$ 7% reduction, $P<0.05$) than the sham-operated control group receiving 1 ml of ethanol 50%. The level of mucosal damage induced by aspirin (200 mg kg^{-1} in 0.2 M HCl, 4 ml kg^{-1}, p.o.) was lower than that induced by ethanol 50%. Nevertheless, conscious PPVL animals again exhibited substantially less mucosal damage (74 $\pm$ 5% reduction, $P<0.05$) than that present in sham-operated controls (Figure 2). The gastric emptying (3 h) of a suspension containing phenol red and aspirin was similar in both sham-operated and PPVL rats (79 $\pm$ 3 vs 72 $\pm$ 3% respectively, $n=4$ both), thus suggesting that differences in the level of injury were not due to variations in the period of time during which the mucosa was in contact with the damaging agent.

Table 1 Oligonucleotides primers used in RT–PCR of eNOS, nNOS, iNOS and cyclophilin

Target gene	*Sequence 5′-3′*	*Annealing T*[a]	*No. cycles*	*Product size*
eNOS	TAC GGA GCA GCA AAT CCA C (sense) CAG GCT GCA GTC CTT TGA TC (antisense)	58°C	38, 40, 42	819 bp
nNOS	ATC TCA GAC CTG ATT CGA GGA GG (sense) ACT GTT GAG GAT GCT CAG CAC AG (antisense)	55°C	30, 32, 34	513 bp
iNOS	AGC ATC ACC CCT GTG TTC CAC CC (sense) TGG GGC AGT CTC CAT TGC CA (antisense)	64°C	34, 36, 38	388 bp
Cyclophilin	CGT CTG CTT CGA GCT GTT TG (sense) GTA AAA TGC CCG CAA GTC AA (antisense)	60°C	20, 24, 28	463 bp

Bp: base pairs

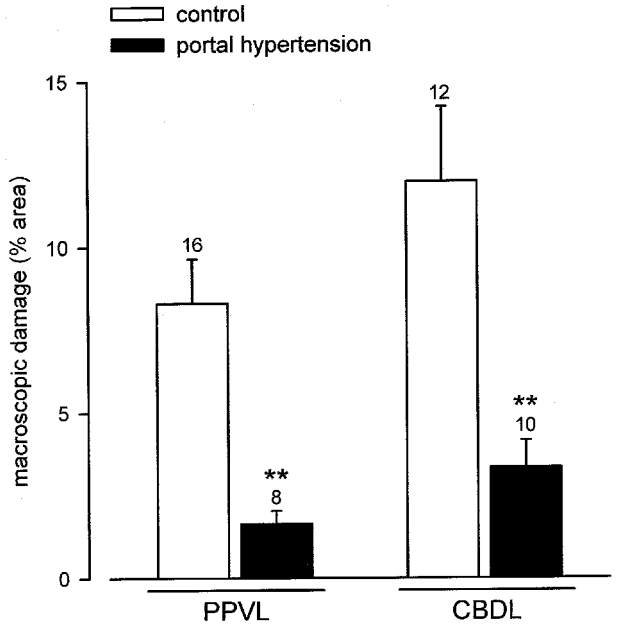

Figure 1 Gastric damage induced by ethanol 50% (1 ml, p.o.) in conscious rats with partial portal vein ligation (PPVL), common bile duct ligation (CBDL) or the corresponding sham operations. Results, shown as the percentage of the mucosal area exhibiting macroscopically assessed damage, are expressed as mean ± s.e.mean of n (above columns) experiments. Significant difference from the respective control sham-operated group is shown as $^{**}P<0.01$ (ANOVA + Student-Newman-Keuls test).

Anaesthetized animals In pentobarbital-anaesthetized animals the presence of absolute ethanol in the gastric chamber induced levels of damage similar to those of 50% ethanol (1 ml) in conscious rats while no differences between PPVL, CBDL and their respective control groups were observed. Furthermore, PPVL rats anaesthetized with pentobarbital did not exhibit any diminution in the degree of injury generated by oral administration of absolute ethanol (Table 2). However, when ketamine was employed as the anaesthetic agent, the level of gastric injury induced by 100% ethanol (p.o) was significantly ($P<0.05$) lower in the PPVL group than that observed in sham-operated animals (Table 2).

Effects of inhibition of NO and prostaglandin synthesis on gastric damage in conscious animals Pre-treatment with L-NAME (3 mg kg^{-1} i.p.) did not modify the level of gastric damage exhibited by sham-operated animals receiving 1 ml 50% ethanol. However, it significantly impeded the protection against the damaging effects of this agent in PPVL conscious rats (Figure 3). L-NAME (3 mg kg^{-1} i.p.) also increased the level of damage induced by aspirin in PPVL conscious rats without affecting that present in sham-operated animals (Figure 4).

Subcutaneous indomethacin did not modify the damaging effects of 50% ethanol either in control or PPVL rats when administered at 2 mg kg^{-1} (8.1 ± 2.1 and 1.1 ± 0.5% of mucosal area, $n=7$ and $n=8$ respectively, $P<0.05$) but both responses were similarly augmented by pre-treatment with 5 mg kg^{-1} of this NSAID (20.4 ± 2.5 and 13.3 ± 4.6% of mucosal area, $n=5$ and $n=4$ respectively, $P<0.05$).

Bleeding studies

As shown in Figure 5 there were no differences in aspirin induced gastric bleeding between PPVL and sham-operated animals. However, the level of macroscopic gastric damage induced by aspirin (200 mg kg^{-1} in 0.2 M HCl, 4 ml kg^{-1}, p.o.) was significantly lower in PPVL rats (40 ± 8% of sham-operated response, $P<0.05$), thus indicating that blood loss per area of injury was significantly greater in the portal hypertensive group. As shown in Table 3, there was a significant increase in prothrombin time and thrombin time in PPVL rats compared to that present in sham-operated control animals.

Gene expression of NOS isoforms

As shown in Figure 6, the semi-quantitative analysis of eNOS, nNOS and iNOS mRNA levels by RT–PCR showed that the expression of iNOS in both gastric mucosa and smooth muscle was increased about 4 fold in PPVL rats compared to that in sham-operated rats. Expression of eNOS was only detected by RT–PCR in gastric smooth muscle and no significant differences were observed between PPVL and sham-operated rats. Again, no obvious differences in the levels of nNOS expression in the gastric mucosa or smooth muscle were observed between PPVL and sham-operated rats. Parallel PCR reactions with primers specifically designed for cyclophilin mRNA were performed as a control for the amount of RNA and, as shown in Figure 6, the amounts of the resulting products were the same in all the tissues compared.

Discussion

The present results demonstrate that conscious rats with chronic portal hypertension display an enhanced resistance to

acute gastric damage. This increased resistance of the mucosa to injury is not singular to the experimental model used insofar that similar responses have been observed in portal hypertension induced by both partial portal vein ligation and bile duct ligation. Furthermore, this protection is not limited to a particular injurious agent. Ethanol is a direct topical gastrolesive agent that acts very rapidly, whereas the damaging action of aspirin has a systemic component, takes several hours to appear and depends partially on the presence of acid. Thus, the finding that both ethanol- and aspirin-induced damage are significantly reduced in conscious chronic portal hypertensive animals implies the existence of a protective mechanism which substantially increases the resistance of this gastric mucosa against a wide range of injurious stimuli.

Our results thus challenge previous studies reporting a reduced resistance to gastric injury in portal hypertension (Beck *et al*., 1992; Ferraz *et al*., 1997; Sarfeh *et al*., 1983; 1988). However, the experimental conditions in these prior investigations could help to explain this discrepancy. For instance, there are important differences among the animal models used to induce portal hypertension. Most previous studies have been carried out employing a two-step complete portal vein occlusion (Sarfeh *et al*., 1983; 1988), a model that does not mimic the clinical situation in patients with cirrhosis, where a rapid and complete occlusion of the portal vein does not occur. Experimentally, the increase in gastric blood flow observed in this model (Piqué *et al*., 1988) is similar to that appearing in PPVL rats (Piqué *et al*., 1990). However, the portal, splacnic and mesenteric vein congestion promoted in the former model is much more pronounced. Such congestion impairs oxygenation of the gastric mucosal surface and subsequently causes alterations in the gastric mucosal barrier, resulting in a decrease in mucosal potential difference (Sarfeh *et al*., 1989). This is not the only difference between the two models, an enhanced lipid peroxidation

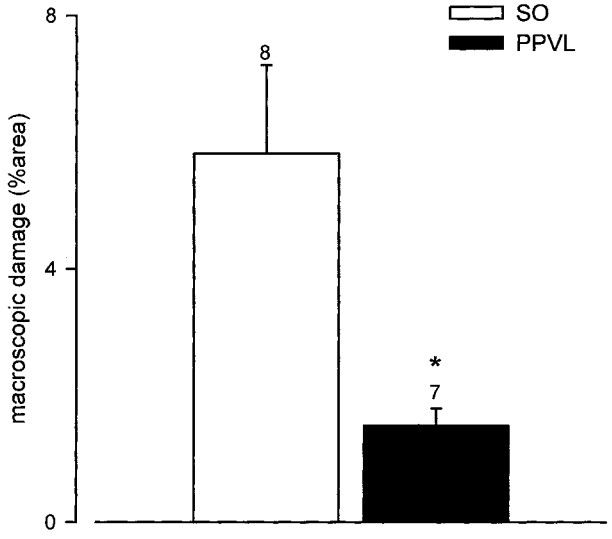

Figure 2 Gastric damage induced by aspirin (200 mg kg^{-1} in 0.2 N HCl) in conscious rats with partial portal vein ligation (PPVL) or sham operation (SO). Results, shown as the percentage of the total mucosal area that exhibited macroscopically assessed damage, are expressed as mean ± s.e.mean of *n* (above columns) experiments. Significant difference from the control sham-operated group is shown as $*P<0.05$ (Student's *t*-test).

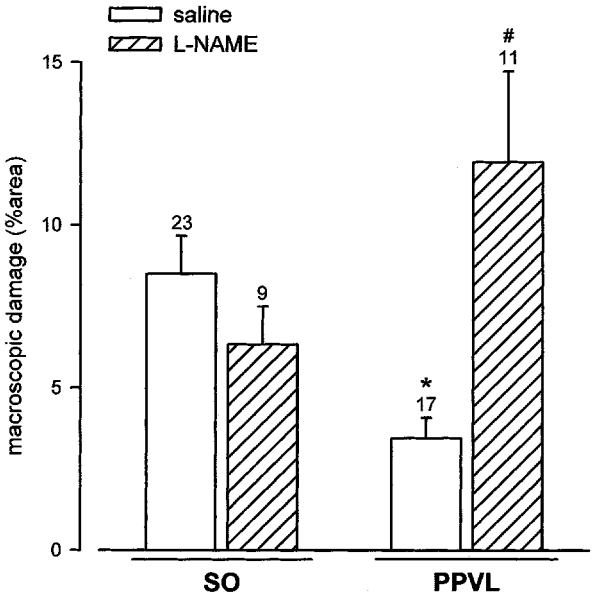

Figure 3 Effects of N^G-nitro-L-arginine methyl ester (L-NAME, 3 mg kg^{-1}, i.p.) on gastric damage induced by ethanol 50% in conscious rats with partial portal vein ligation (PPVL) or sham operation (SO). Results, shown as the percentage of the total mucosal area that exhibited macroscopically assessed damage, are expressed as mean ± s.e.mean of *n* (above columns) experiments. Significant difference is shown as $*P<0.05$ vs sham-operated saline-treated group, and as $\#P<0.05$ vs PPVL saline-treated group (ANOVA + Student-Newman-Keuls test).

Table 2 Macroscopic gastric damage induced by 100% ethanol, applied on the gastric chamber or orally administered, in pentobarbital or ketamine anaesthetized rats

	Pentobarbital		*Ketamine*
Ethanol 100%	*Gastric chamber*	*Oral administration*	*Oral administration*
PPVL			
Sham-operation	12.8 ± 2.4 (8)	11.9 ± 4.9 (3)	5.5 ± 1.2 (15)
Portal hypertension	13.3 ± 4.2 (8)	13.4 ± 6.7 (5)	1.8 ± 0.4 (12)*
CBDL			
Sham-operation	18.1 ± 4.7 (8)		
Portal hypertension	20.6 ± 5.2 (7)		

Results, shown as the percentage of the mucosal area exposed to ethanol that exhibited macroscopically assessed damage, are expressed as mean ± s.e.m. (*n* experiments). PPVL: partial portal vein ligation, CBDL: common bile duct ligation. Significant difference from the corresponding control sham-operated group is shown as $*P<0.05$ (Student's *t*-test).

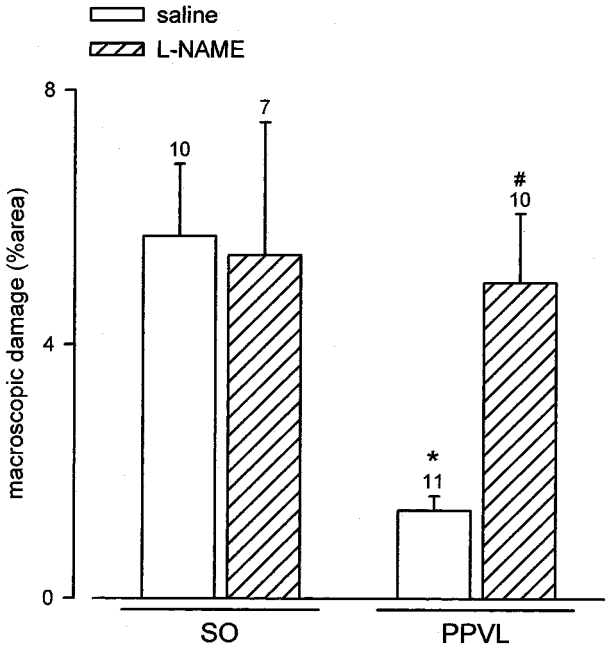

Figure 4 Effects of N^G-nitro-L-arginine methyl ester (L-NAME, 3 mg kg^{-1}, i.p.) on gastric damage induced by aspirin (200 mg kg^{-1} in 0.2 N HCl) in conscious rats with partial portal vein ligation (PPVL) or sham operation (SO). Results, shown as the percentage of the total mucosal area that exhibited macroscopically assessed damage, are expressed as mean±s.e.mean of n (above columns) experiments. Significant difference is shown as $^{**}P<0.05$ vs sham-operated saline-treated group, and as $\#P<0.05$ vs PPVL saline-treated group (ANOVA+Student-Newman-Keuls test).

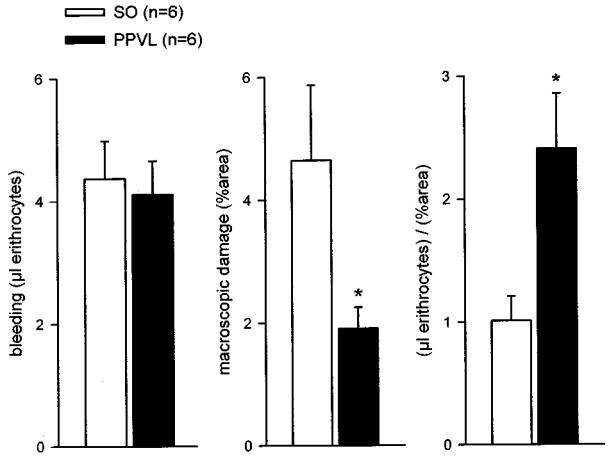

Figure 5 Gastric bleeding, mucosal damage and bleeding per area of injury induced by aspirin (200 mg kg^{-1} in 0.2 N HCl) in conscious rats with partial portal vein ligation (PPVL) or sham operation (SO). Results, shown as the volume of red blood cells in the gastric lumen or the percentage of the total mucosal area showing macroscopically assessed damage, are expressed as mean±s.e.mean of six experiments. Significant difference from the control sham-operated group is shown as $^{*}P<0.05$ (Student's t-test).

having recently been shown to be consequential of increased production of free oxygen radicals in the gastric mucosa of rats with complete portal occlusion (Tarnawski *et al*., 1999). The use of pentobarbital anaesthesia could be another experimental element that justifies the discrepancy between

Table 3 Coagulation results from blood of rats with partial portal vein ligation (PPVL) or sham operation

	Sham-operated	*PPVL*
Activated partial thromboplastin time	15.95±0.59 (8)	16.81±0.85 (8)
Prothrombin time	12.64±0.08 (9)	13.78±0.35 (11)**
Thrombin time	47.21±2.10 (11)	55.64±1.51 (8)**

Results (in seconds) are expressed as mean±s.e.m. (n experiments). Significant difference from the respective Sham-operated value is shown as $^{**}P<0.01$ (Student's t-test).

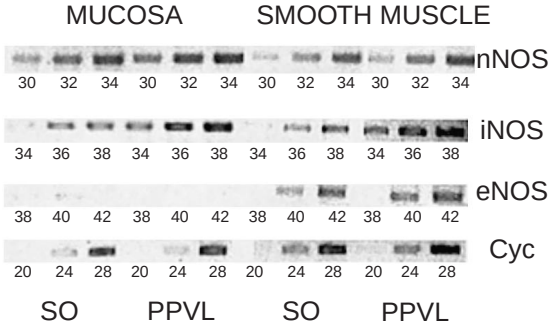

Figure 6 RT-PCR of eNOS, iNOS, nNOS and cyclophilin in gastric mucosa and smooth muscle from rats with partial portal vein ligation (PPVL) or sham operation (SO). Samples were taken after different cycle number for semi-quantitative analysis.

this study and previous reports of biliary cirrhosis increasing susceptibility to rat mucosal injury (Beck *et al*., 1992; Ferraz *et al*., 1997). Although such an augmented sensibility to damage was not noted in our experiments, the enhanced resistance to noxious agents observed in PPVL and CBDL conscious rats was not present when the aforementioned anaesthetic was used. The fact that the results obtained with both intact animals and the gastric chamber are similar shows that the diminished resistance to damage is related to the use of pentobarbital and is not a result of any surgical manipulation. In addition, the finding that the changes provoked by pentobarbital were not present when it was substituted by ketamine suggests that they are characteristic of the drug rather than a condition of general anaesthesia. There are important vascular effects of pentobarbital in portal hypertensive and cirrhotic rats; in particular there is evidence of alterations of basal hemodynamic parameters (Lee *et al*., 1985; 1986) as well as changes in the responses to different vasoactive agents (Kirstetter *et al*., 1996; Lee *et al*., 1991). Especially relevant is the abolition by pentobarbital of gastric hyperemia in PPVL and CBDL rats and the subsequent reduction of their gastric blood flow to that of control animals (Lee *et al*., 1985; 1986), an effect that does not occur when ketamine is employed (Albillos *et al*., 1992; Casadevall *et al*., 1993).

Gastric mucosal integrity depends on the combined action of three vasoactive agents: neuropeptides released from afferent neurons, prostaglandins and nitric oxide (Whittle, 1994). No major alteration in the afferent neuron function has been reported in portal hypertensive rats (Fernández *et al*., 1994). The influence of portal hypertension on endogenous prostaglandin synthesis is not clear, and both increase

and reduction of prostaglandin levels have been reported (Arakawa *et al*., 1987; Casadevall *et al*., 1993; Saperas *et al*., 1990). In the present study, pre-treatment with 2 mg kg^{-1} of indomethacin did not modify the level of damage either in control or PPVL rats but, when the dose of indomethacin was increased to 5 mg kg^{-1} – a dose which totally inhibits the gastric synthesis of prostaglandins – a significant increase in the level of ethanol-induced damage was observed in both groups. Yet, even under these circumstances, the gastroprotection induced by portal hypertension was patent, thereby indicating that the integrity of the portal hypertensive mucosa depends on prostaglandin synthesis to a similar extent to that of control animals.

Augmented levels of nitrites and nitrates have been reported in patients with cirrhosis (Guarner *et al*., 1993; Marsumoto *et al*., 1995). In addition, it has been shown that NO synthesis is increased in portal hypertensive animals, although this NO has been related principally to the changes in vascular reactivity appearing in this condition and not to variations in mucosal susceptibility to damage (Cahill *et al*., 1995; Casadevall *et al*., 1993, García-Pagán *et al*., 1994; Lee *et al*., 1993; Ohta *et al*., 1997; Pilette *et al*., 1996; Pizcueta *et al*., 1992, Wiest *et al*., 1999). We have now demonstrated that the protection against damage induced by ethanol or aspirin in PPVL rats is abolished by pre-treatment with the inhibitor of NO-synthesis L-NAME, at doses that have no effect in control animals. We found no changes in eNOS or nNOS mRNA in the gastric mucosa of PPVL animals and only a very small increase in iNOS mRNA in both the mucosal and muscle layer of the stomach. These results are in accordance with previous evidence provided by ourselves and other groups which shows no increment in constitutive or inducible NOS activity in gastric samples from PPVL rats *ex vivo* (Fernández *et al*., 1995; Kanwar *et al*., 1996). Thus, the origin of the NO mediating the gastroprotection observed in portal hypertension seems due to factors increasing the activity of NOS that are only present *in vivo*. In this context, increased plasma levels of several receptor-dependent NOS activating agents have been reported in portal hypertension (Asbert *et al*., 1993; Bendtsen *et al*., 1991; Henriksen *et al*., 1980). Furthermore, a recent study has shown that the increase in NO synthesis in the mesenteric vasculature of cirrhotic rats is only detectable in the presence of shear stress (Wiest *et al*., 1999). The increased function of NO in protecting the gastric mucosa of portal hypertensive animals may be the result of changes in calcium concentration induced by these or other physiological factors and, therefore, would not be observed in tissue homogenates.

Increases in *ex vivo*-measured constitutive NO-synthase activity have, however, been found in rats with complete portal vein ligation (Ohta *et al*., 1997). There is growing opinion that small changes in the concentration of NO generate important variations in its effects (Colasanti & Suzuki, 2000), including those it has on tissue integrity (Whittle, 1994; Calatayud *et al*., 2000). It is thus plausible that conflicting evidence about the influence of NO on portal hypertension is consequential of minor differences in the degree of increase of this endogenous mediator. Therefore, higher amounts of NO released following complete occlusion of the portal vein may be involved in direct toxic effects (Ohta *et al*., 1997). However, NO released in lower concentrations after PPVL could protect the mucosa by modulating local vascular parameters.

Despite a substantially diminished level of aspirin-induced damage, the gastric lumen of portal hypertensive rats contains the same amount of erythrocytes as control animals, thus indicating an augmented bleeding rate per area of injury. It seems that, although the gastric mucosa of PPVL rats is more resistant to acute damage, the lesions appearing in these animals bleed more than those observed in normal mucosa. This augmented bleeding rate may be due to the damage of those dilated mucosal vessels characteristic of portal hypertension (Albillos *et al*., 1992; Panés *et al*., 1994; Tarnawski *et al*., 1988). Indeed, the increased internal radius and thinner wall of these vessels increase the stress on the vascular wall, rendering it more prone to rupture. We have also observed a significant increase in the prothrombin and thrombin times in blood from PPVL rats. However, these changes are very small in magnitude and probably of little relevance in the haemostatic process.

In summary, the present findings indicate that chronic portal hypertension increases the resistance of gastric mucosa to acute gastric damage, an effect that is modulated by anaesthesia and attributable to an augmented synthesis of nitric oxide.

S. Calatayud is the recipient of a fellowship sponsored by Conselleria de Educació i Ciéncia, Generalitat Valenciana. M.C. Ramirez is the recipient of a fellowship sponsored by Ministerio de Educación y Ciencia. This study has been supported by CICYT grants SAF98-0118 and 1FD97-10289.

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(Received September 29, 2000
Accepted October 19, 2000)