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Effects of inhibiting nitric oxide biosynthesis on the systemic and splanchnic circulation of rats with portal hypertension

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1 The effects of inhibiting endogenous nitric oxide (NO) synthesis with N^G-monomethyl-L-arginine (L-NMMA) on the systemic and splanchnic circulation have been investigated in rats with experimental chronic portal hypertension, anaesthetized with ketamine.

2 Portal hypertension was induced by partial portal vein ligation, 2 weeks prior to study. This procedure induced a reduction in systemic arterial blood pressure (MAP), an increase in cardiac output as measured by radiolabelled microspheres, a reduction in peripheral and splanchnic vascular resistance and an increased portal venous inflow (PVI) and portal pressure, as compared to control non-ligated rats.

3 L-NMAA (6.25 and 50 mg kg^{-1}, i.v.) dose-dependently increased MAP, reduced cardiac output and PVI, and increased peripheral and splanchnic vascular resistance. With L-NMMA (50 mg kg^{-1}), PVI and the vascular resistances returned to values comparable to those determined in control non-ligated anaesthetized rats under resting conditions.

4 Porto-collateral resistance was also increased by these doses of L-NMMA, whereas portal pressure was unchanged. The increase in renal blood flow and decrease in renal vascular resistance also seen in portal-hypertensive rats was reversed by L-NMMA (50 mg kg^{-1}).

5 These effects of L-NMMA (50 mg kg^{-1}) were inhibited by prior administration of L-arginine (300 mg kg^{-1}, i.v.).

6 These findings indicate that the chronic hyperdynamic circulatory characteristics following portal vein stenosis can be attenuated by L-NMMA. Thus, the excessive formation of endogenous NO may be implicated in the pathogenesis of the haemodynamic disturbances and splanchnic vasodilatation associated with chronic portal hypertension.

Keywords: Portal hypertension; splanchnic circulation; nitric oxide; N^G-monomethyl-L-arginine (L-NMMA)

Introduction

Chronic portal hypertension is associated with a hyperdynamic circulation, characterized by increased blood flow and reduced vascular resistance in the splanchnic and systemic circulation (Vorobioff *et al.*, 1983; Kravetz *et al.*, 1986). Such a hyperdynamic circulation observed in patients (Lebrec *et al.*, 1983; Bosch *et al.*, 1988) is reproduced in experimental models of portal hypertension such as that induced by chronic partial portal vein stenosis in the rat (Vorobioff *et al.*, 1983; Blanchet & Lebrec, 1982; Benoit *et al.*, 1984; Kravetz *et al.*, 1986). The mechanisms underlying the splanchnic vasodilatation under such conditions may reflect the reduced sensitivity of the vascular tissue to vasoconstrictor mediators (Kiel *et al.*, 1985; Pizcueta *et al.*, 1990) or the release of vasodilator factors, such as glucagon (Benoit *et al.*, 1984; 1986; Kravetz *et al.*, 1988).

The biological activity of endothelium-derived relaxing factor, (EDRF, Furchgott & Zawadzki, 1980; Furchgott, 1984) can be attributed to endogenous nitric oxide (NO) formed by vascular endothelial cells (Palmer *et al.*, 1987; Ignarro *et al.*, 1987; Khan & Furchgott, 1987; Kelm *et al.*, 1988). The biosynthesis of NO from its substrate amino acid, L-arginine (Palmer *et al.*, 1988a) is inhibited by N^G-monomethyl-L-arginine (L-NMMA) in endothelial cells and vascular tissue *in vitro* (Palmer *et al.*, 1988b; Rees *et al.*, 1989a). Furthermore, studies with L-NMMA *in vivo* have implicated NO in the physiological regulation of systemic arterial blood pressure in the rabbit, rat and guinea-pig (Rees *et al.*, 1989b; Compton *et al.*, 1989; Whittle *et al.*, 1989; Aisaka *et al.*, 1989), of peripheral vascular tone in man (Vallance *et al.*, 1989a,b) and of blood flow in the rat gastric, mesenteric and renal vascular beds (Piqué *et al.*, 1989; Gardiner *et al.*, 1990; Walder *et al.*, 1991).

While a reduction in endogenous NO biosynthesis may be involved in the pathogenesis of some forms of hypertension (Moncada *et al.*, 1991), excessive formation of NO may contribute to cardiovascular disease conditions involving reduced vascular resistance. In the present study therefore, the possible contribution of NO to the altered systemic and splanchnic circulation of the chronic portal hypertensive rat induced by partial portal vein ligation has been investigated by use of L-NMMA.

Methods

Animal preparation

The study was performed in male Sprague-Dawley rats, in which portal hypertension was induced by the ligation of the portal vein, as previously described in detail (Chojkier & Groszmann, 1981; Kravetz *et al.*, 1988). In brief, rats were anaesthetized with ketamine (100 mg kg^{-1}, i.m.), the portal vein isolated and a stenosis created by a single ligature of 3-0 silk placed around both the portal vein and 20 gauge blunt tipped needle. The needle was then removed from the ligature, thus leaving a calibrated constriction of the portal vein. The rats were allowed to recover and had free access to water and food until the day of the study, 2 weeks later, when the portal hypertension syndrome had fully developed.

The portal hypertensive animals were divided in 4 groups: a vehicle control group that received an intravenous bolus injection of saline (1 ml; $n = 9$; body weight 352 ± 18 g); a second group that received L-NMMA at a dose of 6.25 mg kg^{-1}, i.v. ($n = 9$; weight 372 ± 14 g); a third group that received L-NMMA at a dose of 50 mg kg^{-1}, i.v. ($n = 8$, weight 347 ± 12 g) and a fourth group in which L-arginine (300 mg kg^{-1}, i.v.; Sigma Chemical Company, Poole, Dorset, prepared as the acetate) dissolved in isotonic saline was

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administered 5 min before administration of L-NMMA (50 mg kg^{-1}, $n = 7$, weight 368 ± 15 g). These doses were chosen from preliminary dose-response studies on the effects of L-NMMA (1.25–100 mg kg^{-1}) on systemic arterial blood pressure (MAP) in portal hypertensive rats anaesthetized with ketamine, as those that gave submaximal and near maximal responses. L-NMMA (6.25 mg kg^{-1}) was the lowest dose that returned MAP to values comparable to those in control non-ligated animals.

In an additional study using control non-operated rats without portal ligation, one group received the vehicle, saline ($n = 8$, weight 368 ± 19 g) while in a further group, L-NMMA (50 mg kg^{-1}, i.v.; $n = 8$, weight 346 ± 14 g) was administered. L-NMMA as the acetate, prepared in the Dept. Medicinal Chemistry, Wellcome Research Labs, was dissolved freshly in isotonic saline before use.

Haemodynamic studies

The techniques used for the haemodynamic measurements have been described previously in detail (Groszmann *et al.*, 1982; Kravetz *et al.*, 1986; Pizcueta *et al.*, 1989). The rats were anaesthetized with ketamine HCl (100 mg kg^{-1}, i.m.). The left ventricle was catheterized via the right carotid artery with PE-50 tubing. Another PE-50 catheter was placed in the left femoral artery for arterial pressure measurements and for blood withdrawal. Through a 2-cm midline incision, the portal vein was catheterized via the ileocolic vein with tip chilled tubing. After verification that free backflow of blood was obtained, the catheter was fixed with cyanoacrylate glue and the abdomen wall was closed with silk sutures. This catheter was subsequently used for portal pressure measurements. Another PE-50 catheter was placed in the right atrium via the left external jugular vein and used for atrial pressure meaurement and drug infusion. All catheters were connected to pressure transducers, calibrated before each experiment, and blood pressures were registered on a multichannel recorder (Lectromed MT6-PX). The zero reference point was established 1 cm above the operating table. Rectal temperature was maintained at 37 ± 0.5°C throughout the study.

After obtaining baseline measurements of resting MAP, right atrial pressure and portal pressure, saline or L-NMMA (1 ml) was administered as a slow bolus injection through the catheter in the internal jugular vein. Ten minutes later, these pressures were measured again and cardiac output and regional blood flows were measured by a radioactive microsphere technique, as previously described (Kravetz *et al.*, 1986; Pizcueta *et al.*, 1989). A reference blood sample was obtained from the femoral artery catheter over a 75 s period at a rate of 1 ml min^{-1} using a continuous withdrawal pump. Approximately 5×10^4 microspheres labelled with ^{141}Ce (15 ± 3 μm diameter; specific activity: 10 mCi g^{-1}; New England Nuclear, Boston, MA, U.S.A.) were injected into the left ventricle, 15 s after beginning the blood withdrawal.

At the end of the experiments, the animals were injected intravenously with saturated KCl. The abdominal organs were dissected, blotted, weighed, cut into small pieces, and placed in counting tubes. The radioactivity (c.p.m.) of each organ was determined in a gamma-scintillation counter (Packard, 800c). The interference of Cr radioactivity (energy window: 240 to 400 keV) was corrected using Cr and Ce standards.

Cardiac output (ml min^{-1}) was calculated as follows:

$$\text{Injected radioactivity (c.p.m.)} \times \frac{\text{Reference blood flow (ml min}^{-1})}{\text{Reference blood radioactivity (c.p.m.)}}$$

Regional blood flows were calculated as:

$$\text{Organ blood flow (ml min}^{-1}) = \text{Organ radioactivity (c.p.m.)} \times \frac{\text{Reference blood flow (ml min}^{-1})}{\text{Reference blood radioactivity (c.p.m.)}}$$

Portal venous inflow (PVI), which represents the total blood flow entering the portal venous system, was calculated as the sum of blood flow to stomach, spleen, small and large intestines, pancreas and mesentery.

Resistance in each vascular bed was calculated from the ratio between perfusion pressure (P) and blood flow (Q) of each vascular territory. For the calculation of total peripheral vascular resistance, P was the value for mean arterial pressure minus the right atrial pressure and Q was the cardiac output, in the calculation of splanchnic vascular resistance, P was the value for mean arterial pressure minus the right atrial pressure and Q was the cardiac output; in the calculation of splanchnic vascular resistance, P was mean arterial pressure minus portal pressure and Q was the PVI. For the calculation of portocollateral resistance, P was portal pressure minus right atrial pressure and Q was the PVI.

Data analysis

All results are expressed as mean ± standard error of the mean. Student's *t* test for paired and non-paired data and the analysis of variance with contrasts were used in the statistical analysis of the results. Significance was taken at $P < 0.05$.

Results

Two weeks after ligation of the portal vein, the chronic portal hypertensive animals under resting conditions exhibited characteristic haemodynamic disturbances. Thus, there was a significantly ($P < 0.05$) decreased mean arterial pressure (MAP), increased cardiac output, and reduced total peripheral vascular resistance with splanchnic vasodilatation, as shown by lowered splanchnic vascular resistance as compared with anaesthetized control non-operated rats (Tables 1 and 2). Renal blood flow was also significantly increased with a

Table 1 Mean arterial pressure (MAP) and portal pressure (PP) under resting conditions and after the intravenous administration of N^G-monomethyl-L-arginine (L-NMMA, 50 mg kg^{-1}) or saline in control and chronic portal hypertensive rats; the effect of pretreatment with L-arginine (L-Arg; 300 mg kg^{-1}, i.v.) on the actions of L-NMMA in the portal ligated rats is also shown

	Control rats				*Portal hypertensive rats*				
	Resting	*Saline*	*Resting*	*L-NMMA*	*Resting*	*Saline*	*Resting*	*L-NMMA*	*L-NMMA + L-Arg*
MAP (mmHg)	129 ± 4	130 ± 41	121 ± 5	147 ± 4*	100 ± 5†	103 ± 5†	99 ± 6†	146 ± 7*	101 ± 5**
PP	6.3 ± 0.3	6.4 ± 0.3	7.3 ± 0.4	7.1 ± 0.4	11 ± 0.5†	11 ± 0.5†	11 ± 0.3†	12 ± 0.5†	11 ± 0.5†

Results, shown as MAP or PP (mmHg) under resting conditions or following saline or drug administration in control non-operated rats or following chronic portal vein ligation, are mean ± s.e.mean of 8–9 rats, where significant difference from corresponding resting values is given as * $P < 0.01$ and from the resting values in control rats as † $P < 0.05$. Inhibition of the effects of L-NMMA by L-arginine is shown as ** $P < 0.001$.

Table 2 Effects of saline or N^G monomethyl-L-arginine (L-NMMA, 50 mg kg^{-1}) on systemic and splanchnic haemodynamics in control and portal hypertensive rats: the effect of pretreatment with L-arginine (L-Arg; 300 mg kg^{-1}, i.v.) on the responses to L-NMMA in the portal ligated rats is also shown

	Control		*Portal hypertensive*		
	Vehicle ($n = 8$)	*L-NMMA* ($n = 8$)	*Vehicle* ($n = 9$)	*L-NMMA* ($n = 8$)	*L-NMMA + L-Arg* ($n = 7$)
Cardiac output (ml min^{-1} 100 g^{-1})	24.3 ± 2	23.6 ± 1.6	34.2 ± 2.9*	22.9 ± 2.4†	35.7 ± 3**
Peripheral vascular resistance (mmHg ml^{-1} min^{-1} 100 g^{-1})	5.5 ± 0.4	6.4 ± 0.6	3.2 ± 0.3*	6.2 ± 0.4†	3.4 ± 0.3*
Splanchnic vascular resistance (mmHg ml^{-1} min^{-1} 100 g^{-1})	36.2 ± 6	43.3 ± 4	14.6 ± 1.5*	29.7 ± 2.3†	18.7 ± 3.7**

Results, shown as the value of each parameter per 100 g body weight following intravenous administration of vehicle (saline) or L-NMMA or L-arginine and L-NMMA in control non-ligated or chronic portal hypertensive rats, are expressed as the mean ± s.e.mean of (n) studies. Significant difference from the corresponding control non-ligated group is shown as * $P < 0.05$ and from the vehicle (saline) group in the portal hypertensive rats as † $P < 0.05$. Significant inhibition by L-arginine of the actions of L-NMMA is shown as ** $P < 0.05$.

decrease in renal vascular resistance (Table 3). Portal venous inflow was likewise significantly elevated, as was portal pressure, while porto-collateral resistance was unchanged (Tables 1 and 3).

Effects of N^G-monomethyl-L-arginine in portal hypertensive rats

In portal hypertensive rats, intravenous administration of L-NMMA (6.25 and 50 mg kg^{-1}) induced a dose-dependent significant increase in MAP with an increase of 47 ± 7 mmHg ($n = 8$) at the highest dose (Figure 1). The change in MAP was observed within 1 min following administration of L-NMMA, reached its maximal values after 5–10 min, and was maintained for the duration of the study. The increase in MAP was accompanied by a decline in cardiac output, which was significant at the higher dose of L-NMMA (Figure 1).

L-NMMA (6.25 and 50 mg kg^{-1}) dose-dependently and significantly increased total peripheral vascular resistance and splanchnic vascular resistance (Figure 2). At the higher dose, L-NMMA caused a significant ($P < 0.05$) reduction in portal venous flow and increase in porto-collateral resistance, but there was no change in portal pressure induced by either dose of L-NMMA (Figure 3). In the renal circulation, L-NMMA (50 mg kg^{-1}) induced a significant decrease in renal blood flow and an increase in renal vascular resistance (Figure 4).

Comparison of the cardiovascular parameters following administration of the low dose of L-NMMA (6.25 mg kg^{-1}), indicated that MAP in these portal hypertensive rats was comparable to those in control animals under resting conditions (127 ± 3 and 129 ± 4 mmHg, respectively $P < 0.05$). Furthermore, following this dose of L-NMMA, cardiac output and total peripheral vascular resistance were likewise returned to values not significantly different from those in control animals under resting conditions.

Following administration of L-NMMA (50 mg kg^{-1}), the values for all the cardiovascular parameters measured in the portal hypertensive rats were not significantly different from those determined in control animals under resting conditions (Tables 2 and 3), except for MAP, porto-collateral resistance and portal pressure which were significantly higher (Tables 1 and 3).

Effects of L-arginine

Pretreatment of the portal hypertensive rats with L-arginine (300 mg kg^{-1}, i.v.) 5 min before L-NMMA (50 mg kg^{-1}), abolished ($P < 0.001$) its actions on MAP and all of the cardiovascular parameters measured (Tables 1, 2 and 3). L-Arginine

Table 3 Effects of saline or N^G monomethyl-L-arginine (L-NMMA, 50 mg kg^{-1}) on portal and renal haemodynamics in control and portal hypertensive rats: the effect of pretreatment with L-arginine (L-Arg; 300 mg kg^{-1}, i.v.) on the responses to L-NMMA in the portal ligated rats is also shown

	Control		*Portal hypertensive*		
	Vehicle ($n = 8$)	*L-NMMA* ($n = 8$)	*Vehicle* ($n = 9$)	*L-NMMA* ($n = 9$)	*L-NMMA + L-Arg* ($n = 7$)
Portal venous inflow (ml min^{-1} 100 g^{-1})	3.9 ± 0.5	3.3 ± 0.3	6.6 ± 0.5*	4.8 ± 0.5†	6.2 ± 0.8**
Porto-collateral resistance (mmHg ml^{-1} min^{-1} 100 g^{-1})	1.9 ± 0.3	2.3 ± 0.3	1.8 ± 0.2	2.7 ± 0.3†	1.9 ± 0.4**
Renal blood flow (ml min^{-1} 100 g^{-1})	3.6 ± 0.3	2.4 ± 0.3†	4.7 ± 0.2*	3.4 ± 0.3†	5.0 ± 0.5**
Renal vascular resistance (mmHg ml^{-1} min^{-1} 100 g^{-1})	37.3 ± 2.6	71.7 ± 9.1†	22.2 ± 1.4*	43.9 ± 2.8†	22.0 ± 0.3**

Results, shown as the value of each parameter per 100 g body weight following intravenous administration of vehicle (saline) or L-NMMA or L-arginine and L-NMMA in control non-ligated or chronic portal hypertensive rats, are expressed as the mean ± s.e.mean of (n) studies. Significant difference from the corresponding control non-ligated group is shown as * $P < 0.05$ and from the vehicle (saline) group in the portal hypertensive rats as † $P < 0.05$. Significant inhibition by L-arginine of the actions of L-NMMA is shown as ** $P < 0.05$.

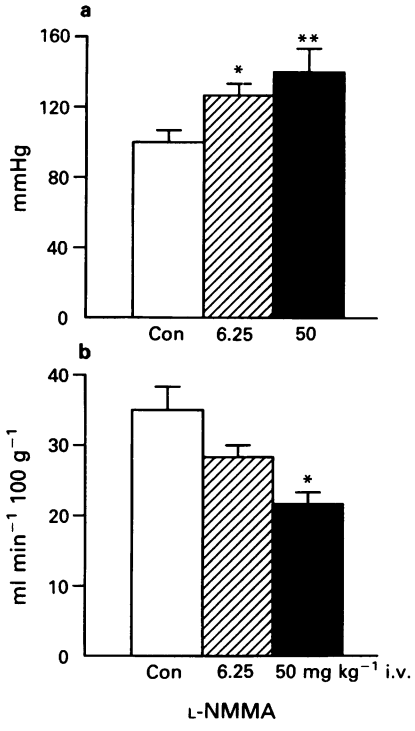

Figure 1 Effects of N^G-monomethyl-L-arginine (L-NMMA, 6.25 and 50 mg kg^{-1}, i.v.) on (a) systemic arterial blood pressure (mmHg) and (b) cardiac output (ml min^{-1} 100 g^{-1} body weight) in the portal hypertensive rat, 2 weeks after portal ligation. Results are shown as the mean of 8–9 experiments in each group, where vertical lines show s.e.mean. Significant difference from the resting values is shown as * $P < 0.05$; ** $P < 0.01$.

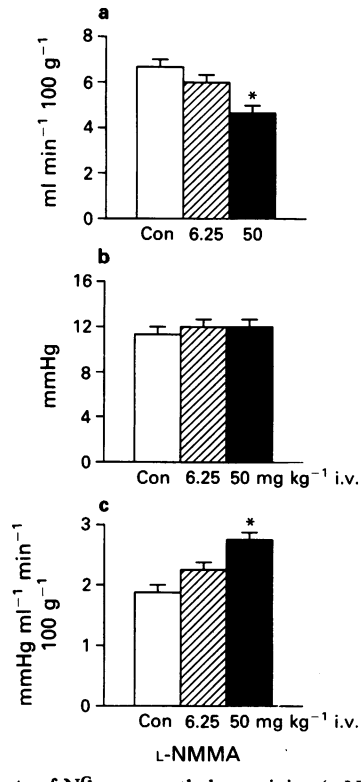

Figure 3 Effects of N^G-monomethyl-L-arginine (L-NMMA, 6.25 and 50 mg kg^{-1}, i.v.) on (a) portal venous inflow (ml min^{-1} 100 g^{-1} body weight) (b) portal pressure (mmHg) and (c) porto-collateral resistance (mmHg ml^{-1} min^{-1} 100 g^{-1} body weight) in the portal hypertensive rat, 2 weeks after portal ligation. Results are shown as the mean of 8–9 experiments in each group, where vertical lines show s.e.mean. Significant difference from the resting values is shown as * $P < 0.05$.

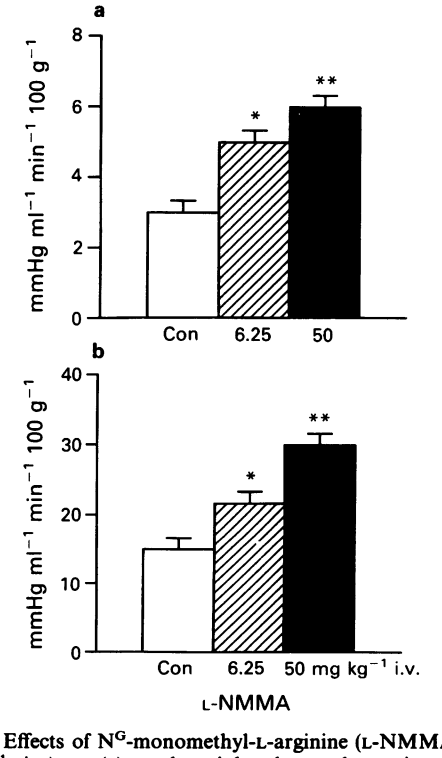

Figure 2 Effects of N^G-monomethyl-L-arginine (L-NMMA, 6.25 and 50 mg kg^{-1}, i.v.) on (a) total peripheral vascular resistance and (b) splanchnic vascular resistance (mmHg ml^{-1} min^{-1} 100 g^{-1} body weight) in the portal hypertensive rat, 2 weeks after portal ligation. Results are shown as the mean of 8–9 experiments in each group, where vertical lines show s.e.mean. Significant differences from the resting values is shown as * $P < 0.05$; ** $P < 0.01$.

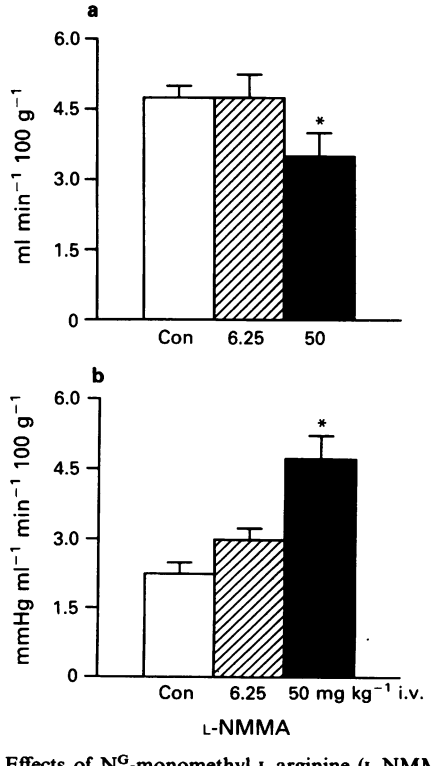

Figure 4 Effects of N^G-monomethyl-L-arginine (L-NMMA 6.25 and 50 mg kg^{-1}, i.v.) on (a) renal blood flow (ml min^{-1} 100 g^{-1} body weight) and (b) renal vascular resistance (mmHg ml^{-1} min^{-1} 100 g^{-1}) in the portal hypertensive rat, 2 weeks after portal ligation. Results are shown as the mean of 8–9 experiments in each group, where vertical lines show s.e.mean. Significant differences from the resting values is shown as * $P < 0.05$.

(300 mg kg^{-1}, i.v.) alone caused an initial inconsistent and transient fall in MAP but there was no significant effect on MAP when measured 5 min after administration, at the time of L-NMMA administration ($P > 0.05$, $n = 5$).

Effects of N^G-monomethyl-L-arginine in control rats

From preliminary dose-response studies with L-NMMA (6.25–100 mg kg^{-1}) in ketamine-anaesthetized control non-ligated rats, a dose of L-NMMA (50 mg kg^{-1}, i.v.) was selected for comparison of its actions on the haemodynamic parameters with those in the portal hypertensive rat.

L-NMMA (50 mg kg^{-1}) significantly increased resting MAP by 26 ± 4 mmHg ($n = 8$, $P < 0.05$) in these control rats, although this was significantly ($P < 0.05$) less than that observed in the portal hypertensive rats with this dose. As with the portal hypertensive rats, L-NMMA did not affect portal pressure in control rats (Table 1). However, unlike the findings in the portal hypertensive rats, the actions of this dose of L-NMMA on cardiac output, portal venous inflow or porto-collateral resistance did not reach statistical significance (Tables 2 and 3). Likewise, the overall changes in total peripheral and splanchnic vascular resistance did not reach significance although L-NMMA did significantly reduce renal blood flow and renal vascular resistance (Tables 2 and 3).

Discussion

Partial ligation of the portal vein in the rat induces an initial elevation of portal pressure and resistance and a decreased venous inflow (Sikuler *et al.*, 1985). However, 8 to 14 days later, extensive collaterals are formed, leading to the porto-systemic shunting of over 90% of the blood entering the portal venous system (Chojkier & Groszmann, 1981). This brings about a decrease of portal resistance to a level comparable to its resting value in non-ligated rats, while a substantial increase in venous inflow contributes to the sustained increase in portal pressure (Vorobioff *et al.*, 1983; Benoit *et al.*, 1984; Sikuler *et al.*, 1985; Kravetz *et al.*, 1986; 1988). The elevated inflow into the portal system is promoted by the accompanying splanchnic and renal vasodilatation, which is reflected by a fall in total peripheral, splanchnic and renal vascular resistance, with a reduction in systemic arterial blood pressure (BP) and increased cardiac output (Groszmann *et al.*, 1982; Sikuler *et al.*, 1985, Benoit *et al.*, 1986; Kravetz *et al.*, 1988). All of these cardiovascular changes were confirmed in the present study in the rat following 2-week partial portal ligation.

The processes underlying the splanchnic vasodilatation in chronic portal hypertension may involve a reduced vascular sensitivity to endogenous vasoconstrictors (Richardson & Withrington, 1976; Kiel *et al.*, 1985; Pizcueta *et al.*, 1990) or an increase in circulating levels of vasodilator factors, possibly due to their decreased hepatic metabolism as a result of porto-systemic shunting (Benoit *et al.*, 1984; 1986; Kravetz *et al.*, 1988). Although glucagon has been implicated (Benoit *et al.*, 1986; Kravetz *et al.*, 1988), studies with infusions of this peptide suggest that it could only account for 30–40% of the observed splanchnic vasodilatation observed in portal hypertensive rats (Benoit *et al.*, 1984; 1986). The present findings using L-NMMA suggest NO is a likely candidate for the mediator of vascular events following chronic portal ligation.

Intravenous administration of L-NMMA dose-dependently elevated BP in the portal hypertensive rat, as observed previously in the conscious or anaesthetized normotensive rat (Compton *et al.*, 1989; Whittle *et al.*, 1989; Gardiner *et al.*, 1990). This was accompanied by a decrease in the cardiac output and an increase in total peripheral, splanchnic and renal vascular resistance to values not significantly different from those observed under resting conditions in control non-ligated rats. In the control non-ligated rats, although BP was increased by L-NMMA, as was renal vascular resistance, the overall changes in total peripheral vascular resistance or total splanchnic resistance did not reach statistical significance. However, recent preliminary studies using similar radiolabelled microsphere techniques in non-operated rats have demonstrated differential changes in the various splanchnic vascular areas following this dose of L-NMMA (Pizcueta *et al.*, 1991). Thus, L-NMMA increased vascular resistance in the stomach, pancreas, spleen and mesenteric bed, but not in the small intestine and colon, indicating different sensitivities to the actions of this NO-synthase inhibitor in these splanchnic vascular beds (Pizcueta *et al.*, 1991). In contrast, in portal hypertensive rats, significant increases in vascular resistance were observed with L-NMMA in all of these vascular beds (Pizcueta *et al.*, 1991), as supported by the current findings of increased total splanchnic resistance. Previous studies with L-NMMA in normotensive chronically instrumented conscious rats have also demonstrated a reduction in regional blood flow, including vasoconstrictor actions on the mesenteric and renal vascular beds (Gardiner *et al.*, 1990), while reductions in blood flow to the gastric mucosa (Pique *et al.*, 1989) and renal cortex (Walder *et al.*, 1991) have been observed in the anaesthetized rat.

In previous studies in this model, a reduction in portal inflow by β-adrenoceptor blockade, or the administration of somatostatin was accompanied by a small but significant fall in the elevated portal pressure (Kravetz *et al.*, 1988; Pizcueta *et al.*, 1989; Piqué *et al.*, 1990). In the current study, the splanchnic vasoconstriction induced by L-NMMA was associated with a reduction in portal venous inflow in the ligated rats, but portal pressure was not significantly modified. This may be the consequence of the concurrent increase in porto-collateral resistance following administration of L-NMMA, which is likely to reflect venoconstriction in the porto-collateral vasculature.

The cardiovascular actions of L-NMMA in the portal hypertensive rats were abolished by pretreatment with L-arginine, as previously demonstrated in normotensive rats (Compton *et al.*, 1989; Whittle *et al.*, 1989; Gardiner *et al.*, 1990). The dose of L-arginine used had itself no significant effect on resting BP, measured 5 min after administration in these portal hypertensive rats. Thus, the mechanism by which L-NMMA could normalize BP, as well as vascular resistance and portal venous inflow, presumably reflects attenuation of the local synthesis of NO from L-arginine, suggesting an exaggerated local production of NO in these vascular beds under such conditions. Furthermore, by comparison with control non-ligated rats, portal hypertensive rats appeared to be more sensitive to the cardiovascular effects of L-NMMA. A detailed comparative evaluation of the sensitivity of these vascular beds to L-NMMA, and other NO synthesis inhibitors under such conditions is hence warranted in future studies. The present findings with L-NMMA also suggest that the porto-systemic collateral vasculature, which is developed from pre-existing venous channels (Vorobioff *et al.*, 1983), is capable of actively synthesizing NO. This contrasts with the findings of a relatively low production of NO by venous tissue under basal conditions (Vallance *et al.*, 1989b).

The factors that regulate substrate availability and enzyme activity involved in endogenous NO synthesis in these vascular beds are not yet fully known. Furthermore, although the calcium-dependent synthase responsible for NO production by endothelial cells is a constitutive enzyme, an inducible enzyme that forms NO from L-arginine and which is also inhibited by L-NMMA, has also been described in these cells (Radomski *et al.*, 1990). An inducible NO synthase, identified originally in the macrophage (Hibbs *et al.*, 1988; Marletta *et al.*, 1988; Stuehr *et al.*, 1989) has also been detected in vascular smooth muscle, lung and liver tissue (Busse & Mulsch, 1990; Knowles *et al.*, 1990; Rees *et al.*, 1990). Furthermore, it has been suggested recently that the vascular formation of NO by this inducible enzyme may contribute to the hyperdynamic

state seen in cirrhosis (Vallance & Moncada, 1991). It will therefore be important to investigate the enzymic characteristics of NO formation in endothelial cells and smooth muscle of the splanchnic vascular beds as well as in the newly developed porto-collateral venous system under the conditions seen in portal hypertension. Should an inducible NO synthase be involved in this systemic and splanchnic vasodilatation, then its selective attenuation would offer a novel and specific approach to the correction of vascular tone and blood flow under such pathological conditions with minimal effects on the physiological actions of NO formed by the constitutive enzyme.

The present findings that the hyperdynamic circulatory characteristics following portal vein stenosis can be attenuated by L-NMMA, thus provide initial evidence that an excessive formation of NO is implicated in the pathogenesis of the haemodynamic disturbances associated with chronic portal hypertension.

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