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Short communication

The role of inducible nitric oxide synthase in vascular hyporeactivity of endotoxin-treated and portal hypertensive rats

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Abstract

The involvement of the inducible nitric oxide (NO) synthase in the vascular hyporeactivity in portal vein-ligated rats was assessed in isolated perfused mesenteric arterial beds. Aminoguanidine, a selective inhibitor of the inducible NO synthase, restored the pressor responses to methoxamine in arteries of endotoxin-treated rats, but was ineffective in hyporeactive portal vein-ligated vessels. N^G-Nitro-L-arginine methyl ester enhanced the responsiveness both in portal vein-ligated and sham-operated rats, without changing the difference between the two groups. These results not only indicate that the inducible NO synthase is not involved in the hyporeactivity to methoxamine in mesenteric arteries of portal hypertensive rats, but also suggest a role for factors other than NO.

Keywords: Acetylcholine; Aminoguanidine; Lipopolysaccharide; Mesenteric arterial bed; N^G-Nitro-L-arginine methyl ester; Portal vein ligation; (Portal hypertensive rat)

1. Introduction

The hyperdynamic state of the splanchnic circulation has been demonstrated to play an important role in the maintenance of elevated portal pressure in portal hypertension (Vorobioff et al., 1983). The cause of the hyperdynamic circulation, however, is still poorly understood. A decreased sensitivity to vasopressors, resulting in low peripheral vascular resistance, has been found both in animal models (Finberg et al., 1981; Kiel et al., 1985; Castro et al., 1993) and cirrhotic patients (Ryan et al., 1993). There is evidence for the involvement of nitric oxide (NO) in the vascular hyporeactivity in portal hypertension, but the source of NO overproduction is still not known (Bomzon and Blendis, 1994). Vallance and Moncada (1991) suggested that the inducible isoform of NO synthase, induced by elevated blood levels of endotoxins due to extensive porto-systemic shunting and/or impaired liver function, might play a role. This hypothesis is supported by the facts that the circulatory derangements in endotoxin shock and portal hypertension are similar and that endotoxemia is often observed in cirrhosis (Vallance and Moncada, 1991). Recently, aminoguanidine has been described as a selective inhibitor of the inducible NO synthase (Hasan et al., 1993). The aim of this study was (i) to investigate the efficacy of aminoguanidine to block the inducible NO synthase in mesenteric arteries of endotoxin-pretreated rats; (ii) to assess the selectivity of aminoguanidine by its effect on the constitutive NO synthase; and (iii) to determine the effect of aminoguanidine on the reactivity to methoxamine in isolated perfused mesenteric vessels of portal vein-ligated rats as an assay for the involvement of the inducible NO synthase.

2. Materials and methods

Male Sprague-Dawley rats weighing 300–350 g were used for mesenteric arterial perfusion. The average body weight of the groups was similar.

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Portal hypertension was induced by partial portal vein ligation (Vorobioff et al., 1983). Briefly, animals were anesthetized by intraperitoneal injecton of pentobarbital sodium. The portal vein was exposed and ligated over a blunt-tipped 21-gauge needle with silk (4-0), leaving a calibrated stenosis of the portal vein. Sham-operated animals were treated in the same manner but without ligation. Perfusion experiments were carried out 10–15 days after surgery.

To test the efficacy of aminoguanidine as an inhibitor of the inducible NO synthase in mesenteric arteries animals were intraperitoneally injected with lipopolysaccharide endotoxin (20 mg kg^{-1}; *Escherichia coli*, serotype 055:B5) or vehicle (saline, 1 ml kg^{-1}) 5 h prior to the experiments. This procedure has previously been shown to result in hyporeactivity to vasoconstrictors both in vivo and in vitro (Julou-Schaeffer et al., 1990), in part due to induction of the inducible NO synthase (Vallance and Moncada, 1991).

Experiments with isolated perfused mesenteric arterial beds were carried out as originally described by McGregor (1965). After intraperitoneal injection of heparin 1000 IU kg^{-1} the animals were killed by cervical dislocation. The superior mesenteric artery was cannulated through a small incision in the abdominal aorta. The mesenteric arterial bed was dissected free from the intestine, then placed on a heating pad and covered with gauze and parafilm. The pad was tilted to facilitate removal of the effluent perfusate. The preparation was perfused at 37° C with oxygenated (95% O_2, 5% CO_2) Krebs solution at a rate of 4 ml/min using a roller pump. The Krebs solution contained (mM) 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 11 glucose and 0.026 EDTA calcium, at a pH of 7.4. Perfusion pressure was measured via a side arm of the arterial cannula by means of a pressure transducer. Vehicle (saline), aminoguanidine (100 μM), N^G-nitro-D-arginine methyl ester (100 μM) and N^G-nitro-L-arginine methyl ester (100 μM) were infused into the perfusion system throughout the experiment using a syringe pump, in each vessel preparation only one of these substances being tested. The experimental protocol involved a 30 min period of equilibration. When determining pressor responses to methoxamine (400 nmol) the preparations were stimulated with the agonist every 5 min until the responses became constant and increases in perfusion pressure above baseline (mm Hg) were calculated. Methoxamine was infused close to the arterial cannula in a volume of 100 μl over a period of 20 s via a roller pump. When relaxation by the NO-dependent vasodilator acetylcholine was tested, mesenteric arterial beds of normal rats were preconstricted with methoxamine 30 μM to 80–100 mm Hg. Since N^G-nitro-L-arginine methyl ester markedly enhanced vasoconstriction, vessels were preconstricted with 10 μM methoxamine in the presence of N^G-nitro-L-arginine methyl ester to a comparable degree. After the perfusion pressure had stabilized, acetylcholine (1 μM) was infused for 3 min by a roller pump. Relaxation was calculated as percentage of the initial perfusion pressure.

All drugs were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Stock solutions were made in distilled water. On the day of the experiment, the final dilutions were prepared in saline. All data are presented as means ± S.E.M. Statistical evaluation was performed with the Mann-Whitney *U*-test, probability values $P < 0.05$ were considered statistically significant.

3. Results

Baseline perfusion pressure was significantly ($P < 0.05$) lower in vessels of endotoxin-treated rats than vehicle-treated rats (9.4 ± 0.3 and 13.3 ± 0.3 mm Hg, respectively; $n = 8$). Aminoguanidine (100 μM) reversed this difference (11.6 ± 0.2 and 13.1 ± 0.2 mm Hg in endotoxin- and vehicle-treated rats, respectively; $n = 8$). Values for baseline perfusion pressure were

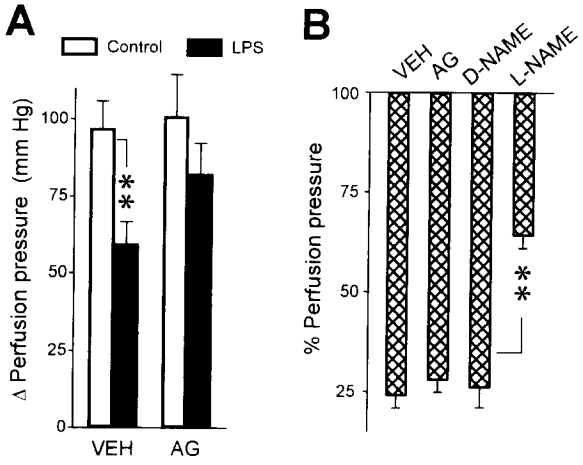

Fig. 1. (A) Effect of aminoguanidine (AG, 100 μM) or its vehicle (VEH, saline) on pressor responses to methoxamine (400 nmol) in isolated perfused mesenteric arteries after intraperitoneal pretreatment with lipopolysaccharide endotoxin (20 mg kg^{-1}) or its vehicle (control; saline, 1 ml kg^{-1}). Note that aminoguanidine reversed the endotoxin-induced hyporeactivity. Data are means ± S.E.M., $n = 8$, ** $P < 0.005$ versus control. (B) Effects of vehicle (VEH, saline), aminoguanidine (AG, 100 μM), N^G-nitro-D-arginine methyl ester (D-NAME, 100 μM) and L-arginine methyl ester (L-NAME, 100 μM) on relaxation by acetylcholine (1 μM) in methoxamine-preconstricted vessel of normal rats. Note that only N^G-nitro-L-arginine methyl ester attenuated the NO-dependent relaxation due to acetylcholine. Data are means ± S.E.M., $n = 6$, ** $P < 0.005$ versus N^G-nitro-D-arginine methyl ester.

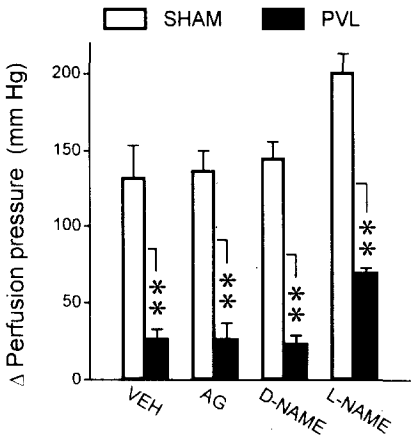

Fig. 2. Effects of aminoguanidine (AG, 100 μM), its vehicle (VEH, saline), N^G-nitro-D-arginine methyl ester (D-NAME, 100 μM) and N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) on pressor responses to methoxamine (400 nmol) in mesenteric arteries of portal vein-ligated (PVL) and sham-operated (SHAM) rats. Note that none of the substances reversed the hyporeactivity due to portal vein ligation. Data are means ± S.E.M., $n = 6$, ** $P < 0.005$ versus SHAM.

likewise significantly lower ($P < 0.005$) in mesenteric arteries of portal hypertensive rats as compared to sham-operated animals (6.3 ± 0.2 and 10.4 ± 0.2 mm Hg, respectively; $n = 6$). Aminoguanidine, N^G-nitro-L-arginine methyl ester or N^G-nitro-D-arginine methyl ester (each 100 μM) did not significantly alter baseline perfusion pressure in vessels of portal hypertensive and sham-operated rats.

Mesenteric arterial beds of endotoxin-treated rats were significantly less reactive to methoxamine, as compared with controls (treated with the vehicle of endotoxin, Fig. 1A). However, when aminoguanidine (100 μM) was infused into the perfusion system no significant difference in responsiveness to methoxamine was observed between the control and endotoxin group (Fig. 1A). At the same concentration, aminoguanidine did not attenuate the relaxation by 1 μM acetylcholine in methoxamine-preconstricted vessels of normal rats (Fig. 1B). N^G-Nitro-L-arginine methyl ester (100 μM) significantly inhibited the relaxation response to acetylcholine, whereas the enantiomer N^G-nitro-D-arginine methyl ester (100 μM) was ineffective (Fig. 1B).

Under control conditions vessel preparations of portal vein-ligated rats exhibited a marked hyporeactivity to methoxamine as compared to sham-operated rats (Fig. 2). Aminoguanidine (100 μM) lacked any effect on pressor responses both in portal vein-ligated and sham-operated rats, leaving the hyporesponsiveness of portal vein-ligated rats unchanged (Fig. 2). N^G-Nitro-L-arginine methyl ester, but not N^G-nitro-D-arginine methyl ester (each 100 μM), potentiated the responses to methoxamine both in portal vein-ligated and sham-operated rats to a similar degree, without reversing the difference between them (Fig. 2).

4. Discussion

In the present study mesenteric arteries of endotoxin-pretreated rats were found to be hyporeactive to methoxamine. In contrast, Mitchell et al. (1993) did not observe hyporeactivity to phenylephrine and other vasoconstrictors in the same vessel preparation, although considerable increases of inducible NO synthase activity were detected in the mesentery. However, these authors used lower doses of endotoxin and of the vasoconstrictors than was done in our study.

Aminoguanidine reversed the hyporesponsiveness to methoxamine induced by pretreatment with endotoxin. The lack of effect of aminoguanidine on the NO-dependent relaxation by acetylcholine indicates that aminoguanidine exerted this effect by blockade of the inducible NO synthase but not the constitutive NO synthase. This is supported by the observation that aminoguanidine had no effect on pressor responses in vessels of vehicle-treated rats. N^G-Nitro-L-arginine methyl ester, a nonselective inhibitor of NO formation, therefore acting both on the constitutive and the inducible isoforms of NO synthase, significantly attenuated relaxation responses to acetylcholine and also potentiated pressor responses in sham-operated animals that did not exhibit hyporesponsiveness. The fact that the enantiomer N^G-nitro-D-arginine methyl ester neither inhibited the acetylcholine-induced relaxation nor faciliated the pressor responses to methoxamine in sham-operated animals suggests that both effects of N^G-nitro-L-arginine methyl ester are specific and therefore due to inhibition of the constitutive NO synthase. The fact that N^G-nitro-L-arginine methyl ester did not completely abolish the relaxation by actylcholine is consistent with a recent study of Adeagbo and Triggle (1993), who found that in mesenteric vessels of rats relaxation by acetylcholine is co-mediated by endothelium-derived hyperpolarizing factor, which is not blocked by N^G-nitro-L-arginine methyl ester. These data indicate that aminoguanidine is a selective inhibitor of the inducible NO synthase induced by endotoxin and agree with previous findings in the rat isolated aorta (Griffiths et al., 1993).

As expected, portal vein ligation also led to hyporeactivity to methoxamine, which, however, was much more pronounced than after pretreatment with endotoxin. Aminoguanidine was ineffective to reverse this, suggesting that the inducible NO synthase is not involved in the hyporeactivity of mesenteric arterial beds due to portal hypertension. Unexpectedly, N^G-nitro-L-arginine methyl ester was also ineffective to restore

the responses to methoxamine in portal vein-ligated rats. N^G-Nitro-L-arginine methyl ester, but not N^G-nitro-D-arginine methyl ester, potentiated the pressor responses, suggesting an effective blockade of basal NO release, but did not diminish the difference in reactivity between sham-operated and portal vein-ligated animals. This is in apparent contradiction to a study of Sieber and Groszmann (1992), who found that N^G-nitro-L-arginine (100 μM), another nonselective inhibitor of NO formation, reversed the hyporeactivity to methoxamine of portal vein-ligated rats in the same vessel preparation as used in this study. These divergent results might possibly be explained by differences in the methods (N^G-nitro-L-arginine was used for inhibition of NO synthesis and pressor responses to methoxamine were determined during periods of 2 min instead of 20 s). Strong evidence against NO as a major cause of the splanchnic vasodilation in portal hypertension is reported by Iwata et al. (1992). After inhibition of NO biosynthesis these authors observed a decrease of blood flow in the splanchnic circulation both in normal and portal vein-ligated animals, but the splanchnic hyperemia of the portal hypertensive, as compared to normal, rats was not reversed.

In conclusion, our results confirm that aminoguanidine is a selective inhibitor of the inducible NO synthase, but do not support the hypothesis that vascular hyporesponsiveness in portal hypertension is due to induction of NO synthase. Furthermore, this study questions the importance of NO as a mediator of hyporesponsiveness to methoxamine in mesenteric arteries of portal vein-ligated rats and suggests that other factors might be responsible for the splanchnic vasodilation in portal hypertension.

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