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Deletion of inducible nitric oxide synthase decreases mesenteric vascular responsiveness in portal hypertensive mice

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Abstract

The effects of pre-hepatic portal hypertension were examined on the responsiveness of aorta and mesenteric artery from wild-type, inducible nitric oxide synthase knockout (iNOS-KO) and endothelial nitric oxide synthase knockout (eNOS-KO) mice. Mice were sham-operated or made portal hypertensive by creating a calibrated portal vein stenosis. Acetylcholine produced marked relaxations in phenylephrine (10 μM) contracted aorta and mesenteric artery from wild-type and iNOS-KO, both sham and portal hypertensive, but relaxations were abolished in vessels from eNOS-KO mice. There were no significant differences between sham and portal hypertensive animals within groups in the effects of acetylcholine. The potency of KCl was significantly increased in aorta and mesenteric artery from eNOS-KO mice. The maximum contraction to the α_1-adrenoceptor agonist phenylephrine was significantly increased in aorta from eNOS-KO, as compared with wild-type mice. There were no significant differences between sham and portal hypertensive animals within each group in contractions of aorta to KCl or phenylephrine. However, in mesenteric artery, although portal hypertension did not change responsiveness in wild-type or eNOS-KO as compared to sham animals, the potency of phenylephrine was significantly reduced in portal hypertensive iNOS-KO mice as compared to shams. Hence, portal hypertension as compared to sham operation did not affect responses to vasoconstrictors in mouse aorta, but in mouse mesenteric artery portal hypertension affected vascular responses in iNOS-KO mice, suggesting that iNOS is involved in the mesenteric vascular response to portal vein ligation.

Keywords: Nitric oxide; iNOS; iNOS knockout; eNOS knockout; NOS-2 knockout; NOS-3 knockout; L-NAME; Portal hypertension; Mesenteric artery, mouse

1. Introduction

The endothelium plays a major role in the local control of blood flow through the release of endothelium-derived factors. Endothelium-dependent relaxation involves nitric oxide (NO) (Furchgott and Zawadzki, 1980; Palmer et al., 1987; Taylor and Weston, 1988), prostacyclin (Moncada and Vane, 1979) and endothelium-derived hyperpolarizing factor (EDHF) (Taylor and Weston, 1988; Komori et al., 1988; Edwards et al., 1998). The role of NO in relaxations to acetylcholine is predominant in large arteries but EDHF has a major role in resistance arteries (Garland and McPherson, 1992; Garland et al., 1995). There are three isoforms of nitric oxide synthase (NOS), NOS-1 (neuronal, nNOS), NOS-2 (inducible, iNOS) and NOS-3 (endothelial, eNOS) (Alderton et al., 2001), but iNOS and eNOS are likely to be of more importance in blood vessels (Briones et al., 2000). Removal of the endothelium (Martin et al., 1986) or addition of nitric oxide synthase inhibitors (Rees et al., 1990) has been shown to increase vasoconstrictor responses. However, attempts to completely remove the endothelium may result in smooth muscle damage, inhibitors of NOS may not be selective for isoforms (NOS-1, iNOS or eNOS), iNOS and eNOS may be involved, and NOS isoforms may not be restricted to the endothelium (Kleschyov et al., 2000; Nelson et al., 2000).

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For these reasons, knockout (KO) technology may give a more useful strategy to investigate the roles of NOS enzymes in modulation of contractions.

Many of the major complications of liver disease result from portal hypertension and portal systemic shunting. The pre-hepatic portal hypertensive rat is a widely used model of human portal hypertension characterised by the development of portal-systemic shunting (Sikuler et al., 1985; Geraghty et al., 1989; Bosch et al., 1992). These shunts develop in response to the increased portal pressure, but a hyperdynamic circulatory state follows, characterised by increased cardiac output and increased splanchnic blood flow. If this increased splanchnic inflow could be prevented, collateralisation and shunting may be prevented or at least diminished. Splanchnic vasodilatation may be due to the increased entry of gastrointestinal vasodilator mediators into the systemic circulation or to diminished responsiveness of the systemic vasculature to endogenous vasoconstrictors. The vascular endothelium may contribute to the hyperdynamic circulation in portal hypertension and cirrhosis (Vallance and Moncada, 1991; Pizcueta et al., 1992; Sieber and Groszmann, 1992; Castro et al., 1993), but there are reports both that inhibitors of nitric oxide synthase (NOSI) failed to restore (Karatapanas et al., 1994) or restored responses to normal in portal hypertensive (Michielsen et al., 1995) and cirrhotic animals (Van Obbergh et al., 1995). Vascular hyporesponsiveness is partially reversed by nitric oxide synthase inhibitors (Martin et al., 1998). Likewise, removal of the endothelium failed to restore (Van Obbergh et al., 1995) or restored (Weigert et al., 1995) responses in portal hypertensive or cirrhotic animals.

The advent of transgenic technology has led us to study a portal vein ligated mouse model of portal hypertension, in which shunting also develops. Hence, there were two aims of this study: to investigate the effects of portal hypertension on vascular responses in wild-type mice; to investigate how iNOS or eNOS deletion affects the response to portal hypertension. To our knowledge, this is the first report of the effects of portal vein ligation on responsiveness of aorta and mesenteric artery from iNOS-KO and eNOS-KO mice.

2. Methods

2.1. Animals

The studies were carried out in accordance with the Declaration of Helsinki and have been approved by the Department of Health and by the RCSI Research Ethics Committee.

Male (except where otherwise stated) wild-type and iNOS-KO or eNOS-KO C57 Black mice (18–28 g) were obtained from Jackson Laboratories (Bar Harbor, ME, USA), and tissues were obtained from portal hypertensive or sham-operated animals. Animals were anaesthetised with ether, a midline incision was made in the abdomen, and the portal vein exposed, a 25-gauge needle was placed alongside the portal vein and a suture was tied around both as close to the liver as possible. The needle was then removed resulting in a calibrated stenosis. The abdomen was then closed and animals were examined 7 days after surgery. In sham-operated animals, the portal vein was exposed but no suture was placed around it. The effectiveness of surgery was assessed by the degree of porto-systemic shunting as measured by the radioactive microsphere technique.

2.2. Radioactive microspheres

Male and female mice were anaesthetised with ether and the spleen was exposed. Radioactive microspheres (^{57}Co, 15 μm diameter, approximately 10,000) were suspended in 10% dextran with 0.01% of the surfactant Tween 80 to prevent clumping and injected in a volume of 0.1 ml. After 3–5 min, animals were killed by cervical dislocation and the lung, liver and kidney removed, chopped up and counted in a gamma scintillation counter. The degree of shunting was assessed from the equation

$$\%\ \text{shunting} = \text{lung c.p.m.} \times 100 \,/(\text{lung c.p.m.} + \text{liver c.p.m.}).$$

Kidney radioactivity was sampled to assess leaching of microspheres.

2.3. Mouse aorta

Animals were killed by CO_2 overdose. Rings of thoracic aorta, 1.5 mm in length, were mounted in a small vessel myograph with 40 μm tungsten wires. Data were recorded on a dual channel electronic display recorder (Myo-Interface Model 400A) and analog acquisition system (MacPacq. MP100, Biopac Systems). Vessels were allowed to equilibrate at 37 °C in Krebs–Henseleit solution (95% O_2/5% CO_2) of the following composition (mM): NaCl 119, $NaHCO_3$ 25, glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.0, EDTA 0.03 and ascorbic acid 0.28. Propranolol (3 μM) was also present to block β-adrenoceptors. The vessel was set to a tension generated at 0.9 times the diameter of the vessel at 100 mm Hg transmural pressure (Mulvany and Warshaw, 1977). Arteries were allowed to equilibrate for 30 min under this passive tension.

In a first set of experiments, tissues were contracted with KCl (40 mM) and acetylcholine (10 μM) was added at the plateau of contraction. After 30-min washing out, KCl was administered cumulatively beginning with 10 up to 120 mM (i.e. vessels were exposed to hypertonic solutions) and the tissues were then washed for 60 min during which the bath was changed every 15 min. A concentration–response curve to phenylephrine (0.01–10 μM) was then performed in a cumulative manner. At the plateau of contraction to the last dose of phenylephrine, cumulative doses of acetylcholine

(0.01–10 μM) were added to the bath to evaluate endothelial function.

In a second set of experiments, arteries from wild-type mice only were contracted with KCl (40 mM). Following a 30-min washout, a concentration–response curve to KCl (10–120 mM) was performed in a cumulative manner in the presence or absence of L-NAME (N^G-nitro-L-arginine methyl ester, 10 μM), added to the bath 20 min before and present throughout the concentration–response curve. Following another 60-min washing-out, concentration–response curves to phenylephrine (0.01–10 μM) were performed in presence or absence of L-NAME (10 μM), added 20 min before the beginning of the phenylephrine concentration–response curve. At the plateau of contraction to the last dose of phenylephrine, cumulative doses of acetylcholine (0.01–10 μM) were added to the bath.

2.4. Mesenteric artery

Animals were killed by CO_2 overdose. Mouse main mesenteric artery was set up as described for mouse aorta, and experiments were carried out exactly as described for the first set of experiments in mouse aorta, except that, following the last dose of phenylephrine, only acetylcholine (10 μM) was added. In studies of mesenteric artery from eNOS-KO mice, both males and females were employed due to limited availability of animals, but no differences in responses were observed between males and females. The sham group consisted of three female and two male, and the portal hypertensive group consisted of five female and two male mice.

2.5. Drugs

Acetylcholine chloride (Sigma, Poole, UK), L-NAME (N^G-nitro-L-arginine methyl ester: Research Biochemicals, Natick, USA), noradrenaline bitartrate (Sigma), phenylephrine hydrochloride (Sigma) and propranolol hydrochloride (Sigma). Drugs were dissolved in distilled water.

2.6. Statistics

Values are mean±S.E.M. from n experiments. Agonist EC_{50} and pD_2 ($-\log EC_{50}$) values as well as maximal contraction were compared between groups by one-way analysis of variance (ANOVA) plus Dunnett's test or Student's t-test for unpaired data. For KCl, additions were non-logarithmic, but for phenylephrine, additions were logarithmic, so that EC_{50} values and pD_2 values and S.E.M. were calculated, respectively, using GraphPadPrism. Statistical and graphical analysis was carried out using Instat for Macintosh and GraphPad Prism for PC. Data used to plot the concentration–response curves are the mean contraction induced at each concentration of the drug, and hence, the maximum response shown graphically may differ from the maximum calculated from individual tissue maxima.

3. Results

3.1. Radioactive microspheres

Degree of porto-systemic shunting was 19.2±7.6% (n=5), 31.7±6.9% (n=5) and 27.5±13.6% (n=5) in portal vein ligated wild-type, iNOS-KO and eNOS-KO, respectively (no significant differences). In sham animals, the degree of shunting was 0.65±0.32% (n=9, data from wild-type, iNOS-KO and eNOS-KO combined).

3.2. Mouse aorta

Acetylcholine induced a concentration-dependent relaxation in aortas from wild-type and iNOS-KO mice precontracted with phenylephrine (10 μM). Acetylcholine had similar maximal effects (Fig. 1) and pD_2 values (6.86±0.19 and 7.12±0.29, respectively, not significant). On the contrary, acetylcholine-induced relaxation was virtually totally blocked in eNOS-KO mouse aorta (Fig. 1). There were no differences between sham and portal hypertensive animals of the same group in the actions of acetylcholine (Fig. 1).

The maximal effect induced by KCl (10–120 mM) was similar in sham animals of the three groups (Fig. 2). The EC_{50} for KCl in iNOS-KO mice aortas was not significantly different from the EC_{50} in wild-type mice (Table 1 and Fig. 2). In sham eNOS-KO mice aortas, the potency of KCl was significantly increased (Table 1 and Fig. 2). There were no differences between sham and portal hypertensive

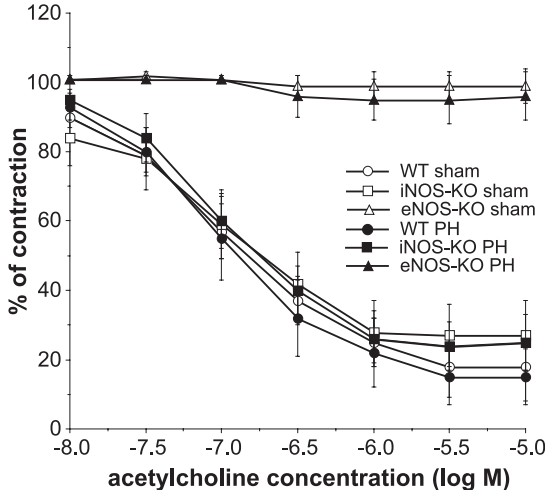

Fig. 1. Acetylcholine (0.01–10 μM)-induced relaxation of phenylephrine-precontracted aorta from wild-type (WT, n=9), iNOS knockout (iNOS-KO, n=6) and eNOS knockout (eNOS-KO, n=7) sham and portal hypertensive (PH) mice. Vertical bars indicate S.E.M. Responses to acetylcholine were abolished in eNOS-KO mice (responses not significantly different from effects of vehicle addition).

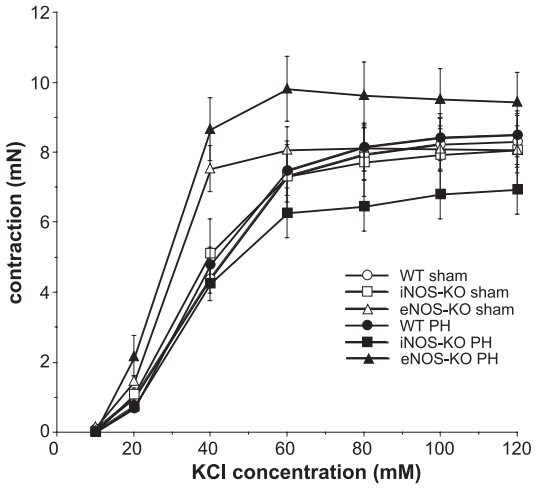

Fig. 2. KCl (10–120 mM)-induced contraction of aorta from wild-type (WT, n=9), iNOS knockout (iNOS-KO, n=6) and eNOS knockout (eNOS-KO, n=7) sham and portal hypertensive (PH) mice. Vertical bars indicate S.E.M. There were no significant differences between sham and portal hypertensive animals of the same group.

animals of the same group in the response to KCl (KCl potency was also increased in portal hypertensive eNOS-KO as compared with other portal hypertensive groups, Table 1 and Fig. 2).

The three groups of sham aortas were similar in terms of potency of phenylephrine (although there was a tendency for increased potency in vessels from eNOS-KO) (Table 1). The maximal response induced by phenylephrine was 1.83±0.27 mN in wild-type. It was significantly increased in eNOS-KO mice aortas (Fig. 3). The maximum response to phenylephrine in eNOS-KO was also significantly larger than in iNOS-KO. Responses to phenylephrine were not significantly affected by portal hypertension in knockout mice as compared with the relevant sham (e.g. wild-type 1.50±0.24 mN, n=9, Fig. 3). However, the difference in potency of phenylephrine did reach significance between wild-type and eNOS-KO portal hypertensive mice (Table 1).

In a second group of experiments, L-NAME (10 μM) abolished acetylcholine-induced vasorelaxation of phenylephrine (10 μM)-contracted vessels from both sham and portal hypertensive wild-type mice (data not shown). In sham animals, L-NAME (10 μM) increased the potency of KCl (Table 1 and Fig. 4) without modifying the maximal contraction (Fig. 4). Mouse aorta from sham animals treated with L-NAME (10 μM) showed an increased potency (Table 1) and maximum contraction (Fig. 4) to phenylephrine. In vessels from portal hypertensive wild-type animals, L-NAME had similar effects to increase potency of KCl (Table 1) without modifying the maximal contraction (Fig. 4). In vessels from portal hypertensive wild-type animals, L-NAME had similar effects to increase maximum contraction to phenylephrine (Fig. 4), but did not increase the potency of phenylephrine (Table 1).

Table 1
Potencies of KCl (EC_{50}, mM) and phenylephrine (pD_2, −log M) in aorta and mesenteric artery from sham and portal hypertensive (PH) rats from wild-type (WT), iNOS-KO and eNOS-KO mice

	Aorta	Mesenteric artery
KCl (mM)		
WT sham	38.5±1.5 (9)	64.0±9.4 (6)
WT PH	38.4±1.3 (9)	45.0±3.8 (5)
WT sham (veh)	37.7±2.0 (6)	
WT sham (L-NAME)	26.6±1.9 (7)$^{+}$	
WT PH (veh)	38.0±1.6 (6)	
WT PH (L-NAME)	29.0±1.1 (6)$^{+}$	
iNOS-KO sham	35.1±1.8 (6)	46.6±3.9 (6)
iNOS-KO PH	35.6±1.5 (6)	53.1±5.5 (7)
eNOS KO sham	26.0±1.0 (7)$^{+}$	17.8±4.6 (5)$^{+}$
eNOS-KO PH	24.9±1.5 (7)$^{+}$	26.5±4.0 (7)$^{+}$
Phenylephrine (−log M)		
WT sham	6.33±0.14 (9)	5.60±0.28 (6)
WT PH	6.32±0.11 (9)	5.53±0.21 (5)
WT sham (veh)	6.37±0.13 (6)	
WT sham (L-NAME)	6.81±0.09 (7)$^{+}$	
WT PH (veh)	6.41±0.17 (6)	
WT PH (L-NAME)	6.51±0.18 (6)	
iNOS-KO sham	6.43±0.12 (6)	5.70±0.09 (6)
iNOS-KO PH	6.22±0.12 (6)	5.12±0.09 (7)*
eNOS KO sham	6.72±0.12 (7)	6.25±0.12 (5)
eNOS-KO PH	6.72±0.10 (7)$^{+}$	6.03±0.11 (7)$^{+}$

Values are mean±S.E.M., with n, the number of experiments in parentheses. Asterisks denote significance of difference between groups (*P<0.05 from effects in sham animal of same group; ^{+}P<0.05 from effects in equivalent WT).

3.3. Mouse mesenteric artery

Acetylcholine (1 μM) produced relaxations of 70.0±12.7% (n=6) and 71.6±8.9% (n=6) in phenylephrine (10 μM) contracted mesenteric artery from wild-type and iNOS-KO mice, respectively. In eNOS-KO mice, acetylcho-

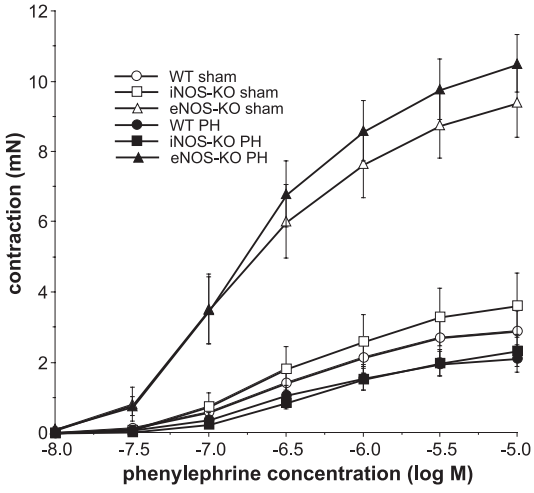

Fig. 3. Phenylephrine (0.01–10 μM)-induced contraction of aorta from wild-type (WT, n=9), iNOS knockout (iNOS-KO, n=6) and eNOS knockout (eNOS-KO, n=7) sham and portal hypertensive (PH) mice. Vertical bars indicate S.E.M. There were no significant differences between sham and portal hypertensive animals of the same group.

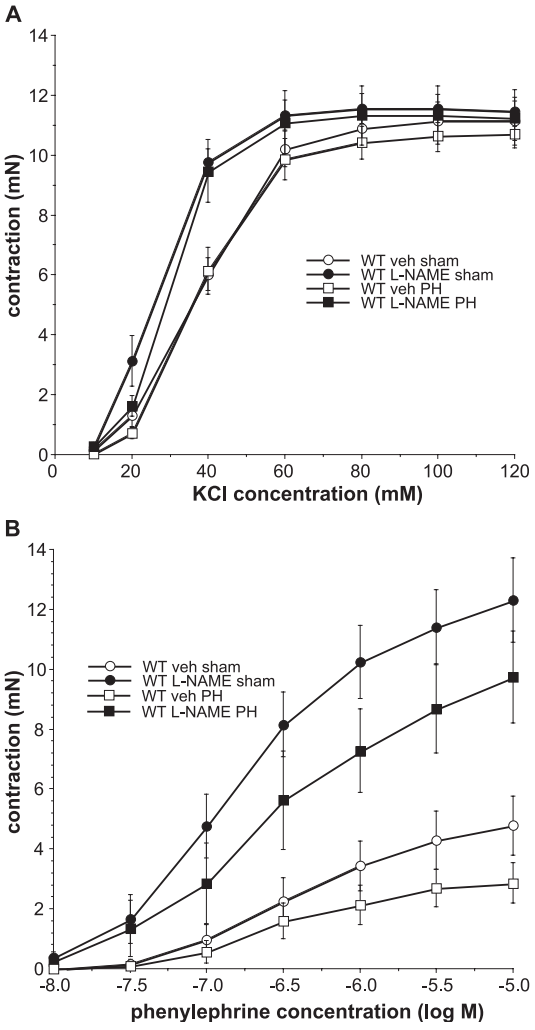

Fig. 4. (A) KCl-induced contraction and (B) phenylephrine-induced contraction of aorta from sham and portal hypertensive (PH) wild-type mice (n=6–7), following exposure to vehicle (veh) or L-NAME (100 μM). Vertical bars indicate S.E.M. L-NAME significantly increased the potency of KCl without altering the maximum contraction in vessels from both sham and portal hypertensive animals. L-NAME significantly increased the potency of phenylephrine only in tissues from sham animals but significantly increased the maximum contraction in vessels from both sham and portal hypertensive animals.

line did not produce a significant relaxation (2.0±1.4%, n=5, P<0.001 from response in wild-type).

The maximal effect induced by KCl (10–120 mM) was not significantly different in sham animals of the three groups (Fig. 5). The EC_{50} for KCl in sham iNOS-KO mouse mesenteric arteries was not significantly different from the EC_{50} in sham wild-type mice (Table 1 and Fig. 5). In sham NOS-3-KO mice mesenteric arteries, the potency of KCl was significantly increased (Table 1). There were no differences between sham and portal hypertensive animals of the same group in the response to KCl (KCl potency was also increased in portal hypertensive eNOS-KO as compared with other portal hypertensive groups) (Table 1 and Fig. 5).

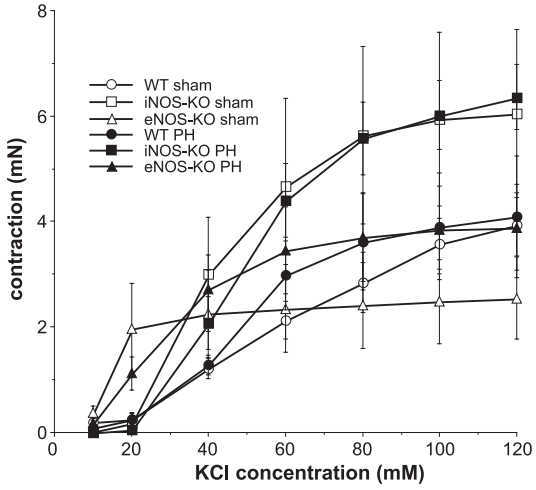

Fig. 5. KCl (10–120 mM)-induced contraction of mesenteric artery from wild-type (WT, n=5–6), iNOS knockout (iNOS-KO, n=6–7) and eNOS knockout (eNOS-KO, n=5–7) sham and portal hypertensive (PH) mice. Vertical bars indicate S.E.M. There were no significant differences between sham and portal hypertensive animals of the same group.

Comparing sham animals, potency and maximum response to phenylephrine were not significantly different between wild-type, iNOS-KO and eNOS-KO sham mice (although there was a tendency for increased potency in vessels from eNOS-KO) (Table 1 and Fig. 6). The difference in potency of phenylephrine did reach significance between wild-type and eNOS-KO portal hypertensive mice (Table 1). Portal hypertension did not affect responses to phenylephrine in mesenteric artery from wild-type or eNOS-KO mice, as compared with sham. However, in iNOS-KO mice, portal hypertension resulted in a significantly reduced potency of phenylephrine in mesenteric artery (5.12±0.09, n=7, P<0.01 from sham) (Table 1 and Fig. 6).

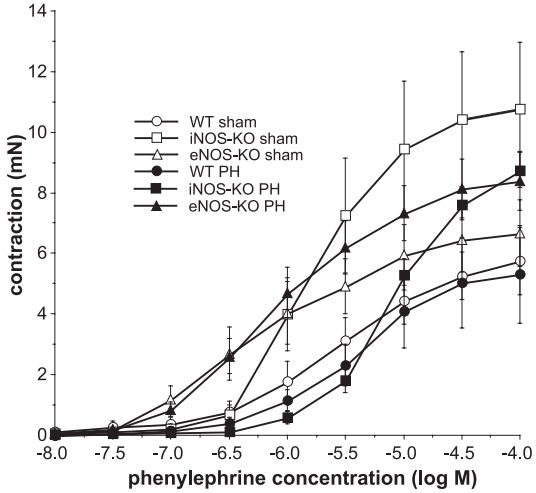

Fig. 6. Phenylephrine (0.01–10 μM)-induced contraction of mesenteric artery from wild-type (WT, n=5–6), iNOS knockout (iNOS-KO, n=6–7) and eNOS knockout (eNOS-KO, n=5–7) sham and portal hypertensive (PH) mice. Vertical bars indicate S.E.M. The potency of phenylphrine was significantly reduced in portal hypertensive as compared with sham iNOS-KO mice.

4. Discussion

In this study, we have examined how deletion of iNOS or eNOS enzymes modulates responsiveness of aorta and mesenteric artery from wild-type and portal hypertensive mice. We will discuss the effects of deletion of iNOS or eNOS on responses in sham animals before discussing the effects of portal hypertension on responsiveness.

Deletion of eNOS, but not iNOS, results in a significant increase in blood pressure (Shesely et al., 1996; Bian et al., 2001). In the present study using in vitro experiments, contractions to KCl were unaffected by iNOS knockout in aorta and mesenteric artery, both in terms of maximum response and potency. However, potency of KCl was significantly increased by eNOS knockout or by L-NAME without change in maximum in both aorta and mesenteric artery. This may suggest that KCl-evoked contractions are modulated to a small extent either by basal or KCl evoked release of eNOS derived NO. In a previous study (Chataigneau et al., 1999), maximal contractions to KCl (60 mM) were unaffected by eNOS deletion, which fits with the present findings of a change in potency only.

The evidence from this study is that relaxations to acetylcholine in mouse aorta and main mesenteric artery involve virtually exclusively eNOS (see also Vandeputte and Docherty, 2002). Other authors found that relaxations to acetylcholine were abolished in aorta, but only partially reduced in first order mesenteric arteries, by eNOS deletion (Chataigneau et al., 1999). Our studies employed main mesenteric artery. In studies of the pulmonary vascular system, deletion of eNOS abolished endothelium-dependent relaxations, although iNOS had a role in modulating basal tone (Fagan et al., 1999).

Although potency of phenylephrine was not significantly changed between sham wild-type and iNOS and eNOS-knockout in both aorta and mesenteric artery, maximum contractions to this agonist were increased to 300% of wild-type by eNOS deletion in aorta but not in mesenteric artery. Previous studies have also shown an increase in the maximal response to phenylephrine due to eNOS deletion or inhibition, but to a smaller extent (20–25% increase: Hussain et al., 1999; Waldron et al., 1999). However, since contractions to KCl were only slightly affected by eNOS deletion, it is unlikely that this modulation of contraction to phenylephrine involves simply basal release of NO. Phenylephrine presumably directly stimulates eNOS in aorta by action at α_1-adrenoceptors and/or α_2-adrenoceptors. Since contractions to phenylephrine involve mainly α_{1D}-adrenoceptors in both rat (Hussain and Marshall, 1997) and mouse (Yamamoto and Koike, 2001) thoracic aorta, it may be difficult to investigate α_1-adrenoceptor actions of phenylephrine on NOS. However, we have recently shown that contractions to noradrenaline are increased by knockout of $\alpha_{2A/D}$-adrenoceptors or by α_2-adrenoceptor antagonists in mouse aorta (Vandeputte and Docherty, 2002), and stimulation of α_2-adrenoceptors may contribute to the actions of high concentrations of phenylephrine.

To confirm the role of eNOS in modulation of contractions to phenylephrine, we assessed the actions of the non-selective NOS inhibitor, L-NAME in aorta. Maximum contraction and potency of phenylephrine were significantly increased by L-NAME (to 6.81 ± 0.09) in aorta from wild-type mice. Although potency of phenylephrine was not significantly increased in aorta from eNOS-KO mice, there was a tendency towards increased potency (6.72 ± 0.12). KCl potency was significantly increased by L-NAME and by eNOS-KO. This suggests that the actions of L-NAME in wild-type are similar to the effects of eNOS deletion on aorta.

In our study, we found unchanged potency and maximum contractions to phenylephrine and KCl in aorta and mesenteric artery from portal hypertensive wild-type animals as compared to sham wild-type. Hence, portal hypertension does not alter vasoconstrictor responsiveness in wild-type mouse aorta and mesenteric artery. However, there was one difference between aorta of sham and portal hypertensive wild-type mice: L-NAME increased the potency and maximum response to phenylephrine in sham, but only the maximum response in portal hypertensive mice. Admittedly, phenylephrine potency in aorta tended to be higher in all eNOS-KO or L-NAME treated groups. This suggests that there is a relatively small increase in potency of phenylephrine following deletion or blockade of eNOS, and significance was reached only in the sham wild-type group following L-NAME, and in the portal hypertensive eNOS-KO mice.

Deletion of iNOS or eNOS did not produce any differences between portal hypertensive and sham animals in responses to vasoconstrictors in mouse aorta. In mesenteric artery, portal hypertension did not alter the response to KCl as compared to the relevant sham in any group of mouse. Likewise, portal hypertension did not alter the potency or maximum response to phenylephrine as compared to sham, in mesenteric artery from eNOS-KO mice. However, the most important finding of this study is that portal hypertension significantly decreased the potency of phenylephrine in mesenteric artery from portal hypertensive as compared to sham-operated iNOS-KO mice.

In mouse mesenteric artery, we have evidence for the involvement of iNOS in the normal vascular response to portal hypertension. Specifically, the lack of iNOS results in reduced potency of α-adrenoceptor agonists in mesenteric artery of portal hypertensive animals, which itself may result in increased splanchnic inflow. Studies of cirrhosis in rat have reported endotoxin-induced increase in mesenteric iNOS activity, and a decrease in hepatic eNOS activity, which may be responsible for increased portal venous inflow and increased hepatic resistance, respectively (Bhimani et al., 2003). Indeed, pulmonary iNOS activity is also reported to increase in portal hypertensive rats and this may contribute to pulmonary pathology (Schroeder et al., 2000).

Since the degree of porto-systemic shunting was unaffected by iNOS-KO, it can be assumed that iNOS is not of critical importance in the development of these shunts, or at least that shunts can occur in the absence of iNOS.

Although it has been generally assumed that responsiveness to vasoconstrictors is diminished in portal hypertension (Bosch et al., 1992; Grose and Hayes, 1992), there are reports of increased (Cawley et al., 1995a,b) responsiveness of rat aorta, rabbit mesenteric veins (Jensen et al., 1987) and rat mesenteric veins (Cummings et al., 1986). In human hepatic arteries, vessels from cirrhotic patients tended to give a larger maximum contraction to vasoconstrictors, although this did not reach significance (Hadoke et al., 1996). There are reports of decreased responsiveness of the in situ mesenteric vascular bed of portal hypertensive rats or cirrhotic rats to noradrenaline, endothelin-1 and angiotensin II (Kiel et al., 1985; Pizcueta et al., 1990; Villamediana et al., 1988, 1989; Yu et al., 1992), and pressor responses to angiotensin II are reduced in bile duct-ligated dogs (Finberg et al., 1981). The most likely reason for this apparent discrepancy between in vitro and in situ studies is that endogenous vasodilators produced in the mesenteric bed enter the systemic circulation through shunt vessels, resulting in increased circulating levels of these vasodilators in portal hypertension (Benoit et al., 1984). These vasodilators act as physiological antagonists of vasoconstrictor responses in vivo, and may cause an adaptive change to occur in the responsiveness of blood vessels to vasoconstrictors. Any altered contractile responsiveness would be revealed in vitro (see Connolly et al., 1999).

Most of the above studies found altered maximum response to vasoconstrictors, whereas the present study of mesenteric artery from portal hypertensive iNOS-KO mice gives a much clearer difference in terms of a reduction in potency. In vascular studies, potency tends to vary less than maximum response between experiments. Loss of potency could be caused by a decreased number of α_1-adrenoceptors or by alteration in the subtype of α_1-adrenoceptors.

Since we began this project, other groups have published results employing this portal vein ligated model in mice lacking iNOS or eNOS. The results reported in eNOS-KO mice have been conflicting: normal development of porto-systemic shunting in both iNOS and eNOS-KO (Iwakiri et al., 2002; Theodorakis et al., 2003; present results), but increased (Iwakiri et al., 2002) or unchanged (Theodorakis et al., 2003) portal pressure and cardiac output in eNOS-KO. In particular, despite iNOS deletion the knockout mice developed a hyperdynamic circulation (Iwakiri et al., 2002; Theodorakis et al., 2003). Hence, despite altered mesenteric vascular responsiveness (present results), the iNOS-KO mice still develop an increased mesenteric vascular inflow, suggesting that compensatory mechanisms come into play. This may suggest that portal vein ligation, leading to porto-systemic shunting and a hyperdynamic circulation, results in increased splanchnic inflow, which normally involves iNOS, but that, in the absence of iNOS, other mechanisms take over such as diminished responsiveness to sympathetic activation. Vasoconstriction of the superior mesenteric artery is an early response to portal vein ligation or even to renal artery ligation (Tsai et al., 2003), triggering upregulation of NOS. An increased splanchnic inflow may be a later response to the haemodynamic changes resulting from portal vein ligation.

In summary, comparing sham animals, eNOS but not iNOS deletion results in increased responsiveness to KCl and phenylephrine in mouse aorta, but only to KCl in mesenteric artery. Portal hypertension does not affect responsiveness to vasoconstrictors in aorta from wild-type, iNOS-KO or eNOS-KO mice. However, portal hypertension decreased potency of phenylephrine in mesenteric artery from iNOS-KO mice. This suggests that iNOS is involved in the normal response to portal vein ligation.

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