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Vascular reactivity in mesenteric resistance arteries following chronic nitric oxide synthase inhibition in Wistar rats

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1 Chronic inhibition of nitric oxide synthase (NOS) induces a sustained hypertension in rats. We studied the effects of chronic inhibition on the *in vitro* vasoreactivity of mesenteric resistance arteries in Wistar rats. We also investigated the effects of acute *in vitro* NOS inhibition in these vessels.

2 Acute NOS inhibition (N^{ω}-nitro-L-arginine, L-NOARG, 10 μM) had no effect on the contractile response to KCl (125 mM), enhanced the response to the phorbol ester, phorbol dibutyrate (1 μM; $69 \pm 9\%$ of KCl response, $n=6$; $38 \pm 7\%$ control, $n=6$, $P<0.05$), increased sensitivity to phenylephrine (EC_{50}: 1.68 ± 0.14 μM, $n=5$; 2.35 ± 0.23 μM control, $n=5$, $P<0.05$) and sodium nitroprusside (SNP; EC_{50} 1.79 ± 0.61 nM, $n=6$; 20.44 ± 6.87 nM control, $n=6$, $P<0.05$) and decreased sensitivity to acetylcholine (EC_{50} 123 ± 12 nM, $n=6$; 45 ± 10 nM control, $n=13$, $P<0.05$).

3 In contrast, contractile responses to KCl (125 mM; 170 ± 12 mN mm^{-3}, $n=10$; 257 ± 21 mN mm^{-3} in control, $n=13$, $P<0.005$) and phenylephrine (maximum response, 30 μM: 169 ± 24 mN mm^{-3}, $n=10$; 295 ± 19 mN mm^{-3} in control, $n=13$, $P<0.001$) were significantly reduced in magnitude following chronic NOS inhibition. Sensitivity to phenylephrine was not significantly altered.

4 The effects of chronic NOS inhibition (N^{ω}-nitro-L-arginine methyl ester, L-NAME, 10 mg kg^{-1} daily for 3 weeks) were similar to those of acute NOS blockade with respect to the relaxant responses to SNP and acetylcholine, and also the contraction in response to protein kinase C activation.

5 Chronic inhibition of NOS significantly increased medial cross sectional area of mesenteric resistance arteries (0.013 ± 0.002 mm^2, $n=7$; 0.009 ± 0.0005 mm^2 control, $n=15$, $P<0.05$).

6 Thus, in contrast to the acute effects of NOS inhibition, chronic NOS inhibition results in a down-regulation of the contractile responses to KCl and phenylephrine in mesenteric resistance arteries, despite an increase in medial cross sectional area. However protein kinase C-dependent contraction remains relatively enhanced. Endothelium-dependent relaxation is reduced and endothelium-independent relaxation is enhanced in a manner similar to the effects of acute NOS blockade.

Keywords: L-NAME; L-NOARG; nitric oxide synthase inhibition; mesenteric resistance arteries; hypertension; phenylephrine; SNP; acetylcholine; hypertrophy

Introduction

Several studies have shown that acute nitric oxide synthase inhibition will enhance the actions of a range of vasoconstrictor agents both *in vitro* and *in vivo* (Conrad & Whittemore, 1992; Manning *et al.*, 1994; Pucci *et al.*, 1994). Chronic inhibition of nitric oxide synthase by administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME) to normotensive rats induces a sustained hypertension (Baylis *et al.*, 1992; Ribeiro *et al.*, 1992; Arnal *et al.*, 1992). It has been suggested that loss of nitric oxide-mediated vasodilatation may be involved in a range of disease states including essential hypertension, atherosclerosis and vasospasm (for review see Moncada *et al.*, 1991a), thus the hypertension which develops as the result of chronic nitric oxide synthase may be a useful model of systemic hypertension. However, little is known about the long-term effects of nitric oxide synthase inhibition on the vasoactive responses of the resistance vasculature. In a previous study using the superior mesenteric artery, Zanachi and colleagues (1993) reported that chronic nitric oxide synthase inhibition diminished responses to contractile agents such as KCl and phenylephrine. In preliminary studies in our own laboratory we have observed that, as expected, the relaxation response to acetylcholine was virtually abolished in the aorta (Henrion *et al.*, 1995); also in the aorta we observed an enhanced sensitivity to the nitrovasodilator, sodium nitroprusside and diminished responses to contractile agents such as KCl and phenylephrine. However, recently the role of nitric oxide in the endothelium-dependent relaxations in smaller resistance type arteries has been questioned. Many studies now show that relaxation may be completely or partially nitric oxide-independent in some vessels (Wu *et al.*, 1993; Vicaut *et al.*, 1994; Waldron & Garland, 1994).

Thus, this study was conducted to investigate the effects of both acute and chronic nitric oxide synthase inhibition on the *in vitro* vascular reactivity of mesenteric resistance arteries from Wistar rats. Contractile responses to KCl, phenylephrine and phorbol dibutyrate were assessed. In addition endothelium-dependent relaxation, in response to acetylcholine and endothelium-independent relaxation, in response to sodium nitroprusside were investigated. In many models of hypertension vascular hypertrophy is observed (Wang & Prewitt, 1990; Griffin *et al.*, 1991). This may have important implications when studying vascular reactivity. In order to allow consideration of this factor we quantified the cross sectional area of the media in mesenteric resistance arteries following chronic nitric oxide synthase inhibition.

Methods

Young male Wistar rats (Iffa-Credo, Lyon, France) weighing 120 to 130 g were given L-NAME (50 mg 100 ml^{-1}) in the drinking water for 3 weeks. This insured a daily intake of L-NAME of 50 mg kg^{-1} (Arnal *et al.*, 1993). In the control

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group rats were given tap water. After 3 weeks rats were anaesthetized (pentobarbitone, 50 mg kg^{-1}, i.v.) and the carotid artery was cannulated (i.d. 0.6 mm) in order to measure blood pressure. The cannula was connected to a pressure transducer (Gould P10EZ, spectramed, Oxnard, CA, U.S.A.) and the signal was displayed on a chart recorder (Gould, Recording Systems Division, Cleveland, OH, U.S.A.). The procedure followed in the care and euthanasia of the study animals was in accordance with the European Community standards on the care and use of laboratory animals (Ministére de l'agriculture, France, authorization nb 00577).

After blood pressure measurement, heparin 1000 iu kg^{-1} was injected through the cannulae. Rat mesenteric artery segments, approximately 200 μm in external diameter, were isolated and immersed in ice cold Tyrode solution of the following composition (in mM): $CaCl_2$ 1.8, KCl 2.7, $MgCl_2$ 0.5, NaCl 136.9, $NaHCO_3$ 11.9, $NaH_2PO_4.H_2O$ 0.04, D-glucose 5.5, N-2-hydroxy-ethylpiperazine-N-2-ethylsulphonic acid (HEPES) 10. The pH of the solution was adjusted to 7.4 with NaOH (1 M).

Segments of second order mesenteric artery (approx. 200 μm external diameter) were trimmed free of fat and adhering connective tissue and mounted in a myograph according to the technique of Mulvany & Halpern (1977). Isometric tension was recorded and collected by a Biopac data acquisition system (Biopac MP 100, La Jolla, CA, U.S.A.) and recorded on a Macintosh Quadra 610 computer (Apple computers, Cupertino, CA, U.S.A.) using the Acqknowledge data acquisition and analysis software (Biopac, La Jolla, CA, U.S.A.). Segments were allowed to equilibrate for 30 min in Tyrode solution, maintained at 37°C and gassed with 95% O_2, 5% CO_2. The Tyrode solution was replaced every 15 min. Each vessel was placed under a stretch equivalent to the *in vivo* arterial blood pessure in each individual rat according to the technique of Mulvany & Halpern (1977). It has been shown that pressure in the mesenteric arcade vessels, which branch off from the vessels used in this study, is approximately 80–90% of the systolic blood pressure (Christensen & Mulvany, 1993); thus the applied level of stretch is assumed to approximate the *in vivo* transmural pressure. Vessels were then allowed to equilibriate for 30 min. The tissue contractility was assessed by exposure to KCl (125 mM); subsequently cumulative concentration-response curves were obtained to phenylephrine (0.1–30 μM). Tissues were allowed to re-equilibrate for 30 min. The mesenteric rings were then used to study the response to one of the following agents: relaxation was assessed in response to either acetylcholine (1 nM–10 μM) {both in the presence and absence of indomethacin (10 μM)} or SNP (1–1000 nM); relaxation responses were obtained following preconstriction with phenylephrine to approximately 50% of the maximum as determined from the phenylephrine response curve. Protein kinase C-dependent contraction was induced with the protein kinase C activator phorbol dibutyrate (1 μM). In a second series of experiments using mesenteric artery segments from control rats the acute effects of nitric oxide synthase inhibition were assessed using the inhibitor N^{ω}-nitro-L-arginine (L-NOARG), which has a very similar pharmacological profile to L-NAME (Wang *et al.*, 1993a,b). Responses to KCl, phenylephrine, phorbol dibutyrate, acetylcholine and SNP were obtained as above and subsequently repeated in the presence of L-NOARG (10 μM).

Contractile responses are expressed in mN tension divided by the amount of tissue (calculated as the product of the vessel circumference, the wall thickness and the segment length) (units: mN mm^{-3}) for each individual segment. Relaxation responses are expressed as a percentage of the phenylephrine-induced tone.

Histology

Segments of artery, adjacent to those used in the functional study, were fixed in 10% formaldehyde in saline and sectioned (10 μM). Morphometric analysis was performed with an automated image processor (NS 15000, Microvision). This system displays the slides onto a screen via a video camera (Hitachi; Tokyo, Japan) mounted on a standard microscope (model 400, Nachet-Vision; Evry, France). Slides were then submitted to an automatic image analysis which outlines the internal and external elastic laminae and calculates the medial cross sectional area (Levy *et al.*, 1988).

Data analysis

The results are presented as mean ± s.e.mean, n = number of rats. EC_{50} (IC_{50} for relaxation) and E_{max} were calculated individually for each concentration-response curve using the equation (Michaelis & Menten, 1913)

$$E = (E_{max} \times C^m)/(EC^m + C^m)$$

where E is the contraction, E_{max} is the maximum contraction, C is the concentration, EC is the EC_{50} (IC_{50} for relaxation) (concentration of agonist required to induce half the maximum response) and m is Hill's coefficient. Statistical analysis was performed by one factor analysis of variance, differences were considered to be significant when $P < 0.05$. Dunnet's test was used for comparisons where appropriate.

Materials

N-2-hydroxy-ethylpiperazine-N-2-ethylsulphonic acid, N^{ω}-nitro-L-arginine methyl ester (L-NAME), N^{ω}-nitro-L-arginine (L-NOARG) and indomethacin were purchased from Sigma (St Louis, MO, U.S.A.). Other reagents were purchased from Prolabo (Paris, France).

Results

L-NAME treatment had no significant effect on rat body weight at the end of the treatment period (395 ± 12 g, n = 25 in control group; 377 ± 11 g, n = 15 in L-NAME group). Mean arterial blood pressure measured in the carotid artery under anaesthesia at the end of the treatment period was found to be significantly increased following chronic L-NAME treatment (120 ± 2 mmHg, n = 25 in control group; 173 ± 6 mmHg, n = 15 in L-NAME group, $P < 0.001$).

Effects of acute nitric oxide synthase inhibition on contractile responses

Acute *in vitro* nitric oxide synthase blockade with L-NOARG had no significant effect on KCl induced contraction (Figure 1a). Following acute nitric oxide synthase blockade the maximum response to phenylephrine was higher than in control vessels, although this effect was not significant (Figure 1b). In addition, sensitivity to phenylephrine was significantly enhanced following acute nitric oxide synthase blockade, illustrated by a lower EC_{50} value in the presence of L-NOARG (1.68 ± 0 14 μM, n = 6) than in control (2.35 ± 0 23 μM, n = 6; $P < 0.05$).

The contraction induced by phorbol dibutyrate was significantly increased following acute nitric oxide inhibition (Figure 2).

Effects of chronic nitric oxide synthase inhibition on contractile responses

Chronic treatment with L-NAME significantly reduced the contractile response to KCl (125 mM) when compared to the response of control vessels (Figure 1a).

The maximum response to phenylephrine was significantly reduced in segments of mesenteric artery from L-NAME-treated rats when compared to control (Figure 1b); the magnitude of the reduction was comparable to the reduction in response to KCl. No significant effect on phenylephrine sensitivity was observed following chronic L-NAME treatment as

shown by the EC_{50} values in arteries from treated (2.42 ± 0.47 μM, $n=10$) and control rats (2.13 ± 0.25 μM, $n=13$).

The contraction induced by phorbol dibutyrate was significantly increased following chronic nitric oxide inhibition (Figure 2). When expressed as absolute values (mN mm^{-3}) this increase was not significant following chronic nitric oxide synthase inhibition; however, if the responses were expressed as a percentage of the potassium-induced contraction, representative of the maximum contractile response of the tissue and thus taking into account the overall reduced contractility, a significant increase in protein kinase C-dependent contraction was observed.

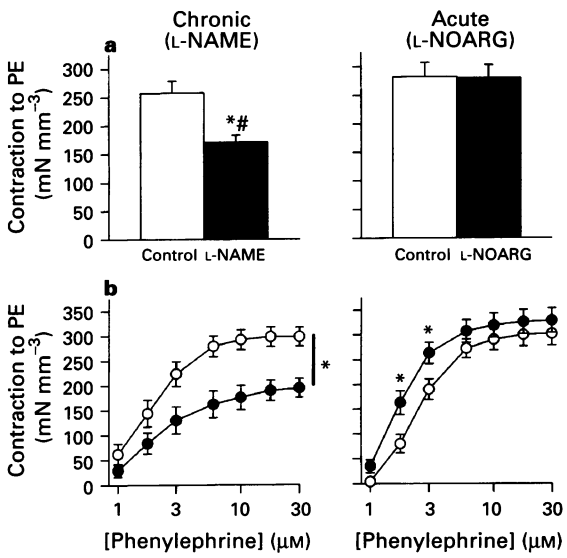

Figure 1 Effects of nitric oxide synthase inhibition on the response to (a) KCl and (b) phenylephrine in rings of mesenteric artery. Open columns/symbols represent control, solid columns/symbols represent chronic (left hand panel, L-NAME) and acute (right hand panel, L-NOARG) nitric oxide synthase inhibition. Data represent mean ± s.e.mean, $n=6-13$. $^*P<0.05$ compared to control. $^{\#}P<0.05$ compared to acute L-NOARG.

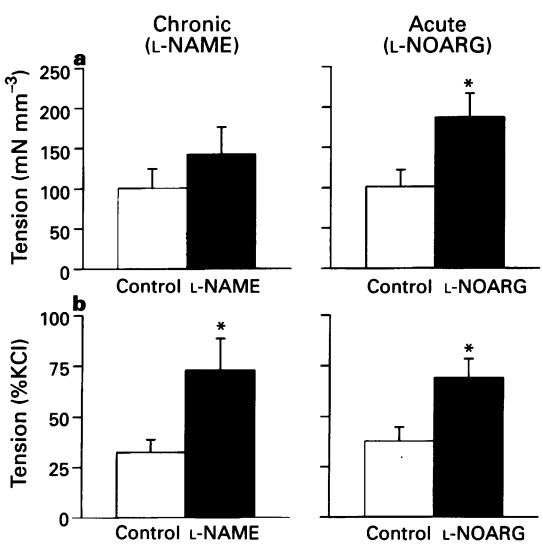

Figure 2 Effects of nitric oxide synthase inhibition on the response to the protein kinase C activator, phorbol dibutyrate, in rings of mesenteric artery. Open columns represent control, solid columns represent chronic (left hand panel, L-NAME) and acute (right hand panel, L-NOARG) nitric oxide synthase inhibition. Contractile responses are expressed in mN mm^{-3} in the upper panel. In the lower panel the responses are expressed as a percentage of the maximum response induced by KCl in order to take into account the overall reduction in contractility of the vessel following chronic NOS inhibition. Data represent mean ± s.e.mean, $n=7-9$. $^*P<0.05$ compared to control.

Effects of nitric oxide synthase inhibition on relaxant responses

The sensitivity to acetylcholine was significantly reduced in the mesenteric artery following acute nitric oxide synthase blockade (EC_{50}: 123 ± 12 nM, $n=5$; 58 ± 14 nM, $n=8$ control, $P<0.001$); however, no significant change in maximum response was observed (Figure 3). Preconstriction levels were comparable in the two groups (52 ± 7% and 54 ± 13% as % of the maximum response to phenylephrine in the control and L-NOARG groups, respectively). Similar effects were observed in vessels from the chronically L-NAME-treated rats when compared to control (EC_{50}: 98 ± 21 nM, $n=10$; 45 ± 10 nM, $n=12$ control, $P<0.05$), whilst the maximum response was not affected (Figure 3). Again there was no significant difference in preconstriction levels between the two groups (48 ± 5% and 54 ± 5% as % of the maximum response to phenylephrine in the control and L-NAME groups respectively). Pre-incubation with indomethacin (10 μM) had no significant effect on the response to acetylcholine either in treated or untreated vessels (EC_{50} values of 56 ± 14 nM, $n=6$ and 105 ± 43 nM, $n=4$ in the presence of indomethacin for the control and L-NAME-treated groups respectively).

Following acute nitric oxide synthase blockade, sensitivity to SNP was significantly increased (EC_{50}: 1.79 ± 0.61 nM, $n=6$;

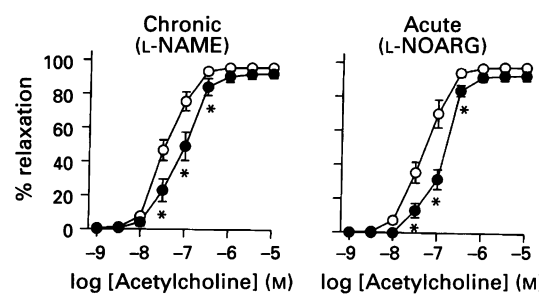

Figure 3 Effects of nitric oxide synthase inhibition on the response to acetylcholine in rings of mesenteric artery. (○) Control; (●) chronic (left hand panel, L-NAME) and acute (right hand panel, L-NOARG) nitric oxide synthase inhibition. Data represent mean ± s.e.mean, $n=6-13$. $^*P<0.05$ compared to control.

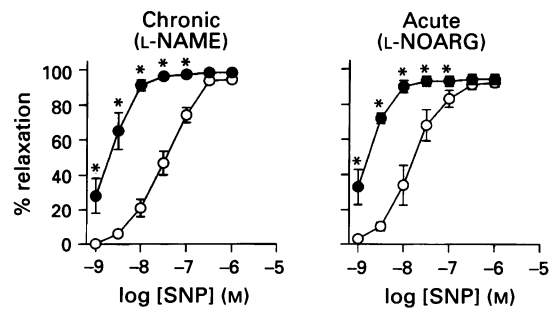

Figure 4 Effects of nitric oxide synthase inhibition on the response to sodium nitroprusside in rings of mesenteric artery: (○) control; (●) chronic (left hand panel, L-NAME) and acute (right hand panel, L-NOARG) nitric oxide synthase inhibition. Data represent mean ± s.e.mean, $n=6-13$. $^*P<0.05$ compared to control.

20.44±6.88 nM, $n=6$ control), whilst no significant change in maximum response was observed (Figure 4). Preconstriction levels were comparable in the two groups (48±6% and 46±9% as % of the maximum response to phenylephrine in the control and L-NOARG groups respectively). Similarly the sensitivity to SNP was significantly increased in the mesenteric artery from the chronically L-NAME treated rats when compared to control vessels (EC_{50}: 2.23±0.61 nM, $n=6$; 27.00±6.07 nM, $n=6$ control), with no significant effect on the maximum response (Figure 4). Again there was no significant difference in preconstriction levels between the two groups (52±4% and 64±8% as % of the maximum response to phenylephrine in the control and L-NAME groups respectively).

Effects of nitric oxide synthase inhibition on vessel structure

Chronic treatment with L-NAME induced a significant increase in the cross sectional area of the media (Figure 5).

Discussion

The aim of this study was to investigate the effects of chronic nitric oxide synthase inhibition on the vasoreactivity of mesenteric resistance arteries.

Chronic inhibition with one of a range of analogues of L-arginine induces a sustained hypertension in rats. This hypertension is thought to be primarily due to the loss of both basal and stimulated nitric oxide production which normally acts to exert a relaxant force on the vasculature (Baylis *et al.*, 1992; Ribeiro *et al.*, 1992; Arnal *et al.*, 1992). However, the precise aetiology of this hypertension has yet to be established. Several studies have been published reporting the effects of concurrent administration of a range of antagonists with nitric oxide synthase inhibitors. The angiotensin II AT_1 receptor antagonist, losartan, has been shown to have no effect (Baylis *et al.*, 1993; Bank *et al.*, 1994), partially reverse (Ribeiro *et al.*, 1992) or completely prevent (Jover *et al.*, 1993; Pollock *et al.*, 1993) the hypertension induced by nitric oxide synthase inhibition. Similarly, endothelin antagonists have produced equally contradictory results (Bank *et al.*, 1994; Richard *et al.*, 1995). These data suggest that hypertension associated with chronic nitric oxide synthase blockade might involve enhancement of peptide-dependent vasoconstrictor tone. Thus, further studies are required to elucidate the precise mechanisms underlying this model and the interaction between the suppression of nitric oxide synthase activity and the enhancement of vasoconstrictor tone.

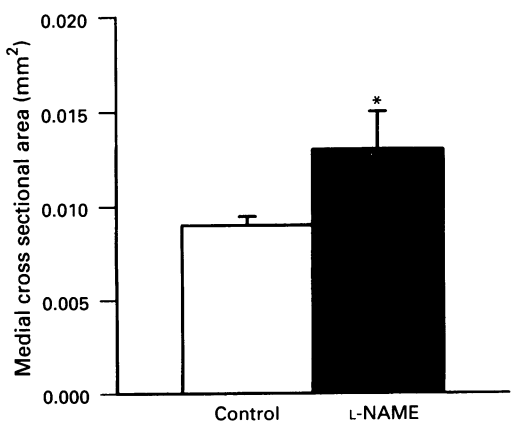

Figure 5 Effects of nitric oxide synthase inhibition on the ratio of media to lumen area in sections of mesenteric artery: Open column represents control; solid column represents chronic (L-NAME) nitric oxide synthase inhibition. Data represent mean ± s.e.mean, $n=8-16$. *$P<0.05$ compared to control.

This study was designed to investigate both the acute *in vitro* and chronic *in vivo* effects of nitric oxide synthase inhibition and the resultant hypertension on vascular reactivity in mesenteric resistance arteries. Acute nitric oxide synthase inhibition had no effect on the response to KCl, increased phorbol dibutyrate-stimulated contraction, resulted in an increased sensitivity to phenylephrine and SNP and decreased the sensitivity to acetylcholine with no effect on the maximum response to endothelium-dependent relaxation. Previous studies have documented the acute effects of nitric oxide synthase inhibition on vasoreactivity in a range of vessels. It has been demonstrated in large conductance vessels such as the aorta, pulmonary artery and superior mesenteric artery that acute nitric oxide synthase inhibition virtually abolishes endothelium-dependent relaxation in response to agonists such as acetylcholine and in response to the calcium ionophore, A23187 (Nagao *et al.*, 1992; Hwa *et al.*, 1994). In contrast, in smaller vessels such as the femoral artery, small mesenteric arteries and terminal arterioles in the rat cremaster muscle a large proportion of endothelium-dependent relaxation is resistant to nitric oxide synthase inhibition, suggesting that the contribution of nitric oxide to endothelium-dependent relaxation is less important in these vessels (Nagao *et al.*, 1992; Vicaut *et al.*, 1994; Hwa *et al.*, 1994; Waldron & Garland, 1994). Furthermore, it has been demonstrated that acute nitric oxide synthase inhibition in aortic rings can enhance the contractile response to agonists such as phenylephrine and other vasoconstrictor agents, and also increase sensitivity to nitrovasodilators such as SNP (Moncada *et al.*, 1991b; Conrad & Whittemore, 1992; Manning *et al.*, 1994; Pucci *et al.*, 1994).

In our study, the increased response to phenylephrine in mesenteric resistance arteries is less dramatic than has been reported in the aorta. Moncada and co-workers reported a 25% increase in maximum response and a 10 fold increase in sensitivity. In contrast, we observed no significant increase in maximum and a 1.4 fold increase in sensitivity. In addition, no significant change in baseline tension was observed when L-NOARG was added to the myograph chamber (data not shown), again this is in contrast to previous reports in both rabbit (Palmer *et al.*, 1988) and rat aorta (Moncada *et al.*, 1991b). Taken together these results support the theory that the role of nitric oxide in smaller resistance type vessels is less important than in the larger conductance arteries. However, acute nitric oxide inhibition did result in an enhanced contractile response to the protein kinase C activator, phorbol dibutyrate. It has previously been reported that nitric oxide induces a reversible inactivation of protein kinase C activity and phorbol ester binding (Gopalakrishna *et al.*, 1993). This indicates that basal production of nitric oxide in the vessel results in a tonic inhibition of protein kinase C, illustrated in our study by an enhanced contractile response to phorbol dibutyrate during chronic nitric oxide synthase inhibition.

The effects of chronic inhibition of nitric oxide synthase on vascular reactivity have not yet been fully investigated. We observed that whilst the effects on the relaxant properties, both endothelium-dependent and -independent, were identical following either acute or chronic inhibition of nitric oxide synthase, the contractile properties of the vessel were notably different. Contraction in response to KCl-induced depolarization or α_1-adrenoceptor stimulation by phenylephrine was reduced in magnitude. Furthermore, sensitivity to phenylephrine tended to be reduced; this is in contrast to the enhanced sensitivity to phenylephrine following acute nitric oxide synthase blockade. Thus, following prolonged inhibition, the acute hypersensitivity to agonist-induced contraction leads to an increased response which subsequently induces a down-regulation of the contractile apparatus, although not sufficiently to normalize blood pressure. The mechanism by which this adaptation occurs is not evident from our study. From our results we can suggest that this adaptation has occurred at two

levels: firstly, the overall contractile response is reduced – illustrated by the comparable decrease in the magnitude of response to both KCl and phenylephrine; and secondly down regulation of receptor-activated pathways may have occurred as the observed acute increase in sensitivity to phenylephrine is reversed in the chronic model.

We also observed an increased medial cross sectional area in the mesenteric resistance artery. This is consistent with previous reports demonstrating arterial hypertrophy in the carotid artery following nitric oxide synthase inhibition (Delacrétaz *et al.*, 1994). The reduction in maximum contractile response to both KCl and phenylephrine is especially important when considered in light of the observed increase in medial cross sectional area, an increased media mass would be expected to result in an increase generation of force. Previous studies in the aorta (Henrion *et al.*, 1995) and superior mesenteric artery (Zanachi *et al.*, 1993) have also demonstrated a decrease in vascular smooth muscle contraction following nitric oxide synthase inhibition. However, in these studies acetylcholine-induced relaxation was also completely inhibited following nitric oxide synthase inhibition. It is interesting to observe that the contractile response is significantly impaired even in a vessel where the agonist-induced endothelium-dependent relaxation is only marginally affected. It is unlikely that the decrease in contractility is simply a response to the hypertension associated with nitric oxide synthase blockade, as we have recently reported that in an angiotensin II based model of hypertension, where similar increases in blood pressure were observed, no significant change in maximum contractile response to KCl or phenylephrine was observed (Dowell *et al.*, 1995).

Following chronic inhibition of nitric oxide synthase the response to protein kinase C activation was not significantly increased compared to control when expressed in absolute values. However, when expressed as a percentage of the KCl-induced contraction, the magnitude of the increase in response was similar to that observed during acute nitric oxide synthase inhibition. Thus, due to the overall decrease in contractility during chronic nitric oxide inhibition, the fact that the response to protein kinase C activation remains enhanced is observed only when expressed relative to the maximum response of the tissue. The observations that the protein kinase C response is enhanced and the medial cross sectional area is increased may be linked to the fact that nitric oxide acts as an inhibitory influence on protein kinase C (Gopalakrishna *et al.*, 1993), angiogenesis (Pipili-Synetos *et al.*, 1994) protein and collagen synthesis in vascular smooth muscle cells (Kolpakov *et al.*, 1995) and smooth muscle cell growth (Garg & Hassid, 1989; Cornwell *et al.*, 1994). If nitric oxide has an inhibitory influence on the activity of protein kinase C, and thus reduces the effect of growth factors acting via protein kinase C, then blockade of nitric oxide synthase would result in vascular smooth muscle cell growth.

In summary, we have reported that, in contrast to the acute effects of nitric oxide synthase inhibition where increased sensitivity to contractile agents is observed, chronic nitric oxide synthase inhibition results in attenuation of the contractile responses in mesenteric resistance arteries, despite an increase in medial cross sectional area. However, relative to the maximum response of the tissue, protein kinase C dependent contraction remains enhanced. Endothelium-dependent relaxation is reduced and endothelium-independent relaxation is enhanced following chronic nitric oxide synthase blockade in a manner similar to the effects of acute nitric oxide synthase blockade.

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