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Alteration of flow-induced dilatation in mesenteric resistance arteries of L-NAME treated rats and its partial association with induction of cyclo-oxygenase-2

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1 We investigated the response to pressure (myogenic tone) and flow of rat mesenteric resistance arteries cannulated in an arteriograph which allowed the measurement of intraluminal diameter for controlled pressures and flows. Rats were treated for 3 weeks with N^G-nitro-L-arginine methyl ester (L-NAME, 50 mg kg^{-1} day^{-1}) or L-NAME plus the angiotensin I converting enzyme inhibitor (ACEI) quinapril (10 mg kg^{-1} day^{-1}).

2 Mean blood pressure increased significantly in chronic L-NAME-treated rats (155 ± 4 mmHg, $n=8$, vs control 121 ± 6 mmHg, $n=10$; $P<0.05$). L-NAME-treated rats excreted significantly more dinor-6-keto prostaglandin $F_{1\alpha}$ (dinor-6-keto $PGF_{1\alpha}$), the stable urinary metabolite of prostacyclin, than control rats. The ACEI prevented the rise in blood pressure and the rise in urinary dinor-6-keto $PGF_{1\alpha}$ due to L-NAME.

3 Isolated mesenteric resistance arteries, developed myogenic tone in response to stepwise increases in pressure (42 ± 6 to 847 ± 10 mN mm^{-1}, from 25 to 150 mmHg, $n=9$). Myogenic tone was not significantly affected by the chronic treatment with L-NAME or L-NAME + ACEI.

4 Flow (100 μl min^{-1}) significantly attenuated myogenic tone by $50 \pm 6\%$ at 150 mmHg ($n=10$). Flow-induced dilatation was significantly attenuated by chronic L-NAME to $22 \pm 6\%$ at 150 mmHg ($n=10$, $P=0.0001$) and was not affected in the L-NAME + ACEI group.

5 Acute *in vitro* N^G-nitro-L-arginine (L-NOARG, 10 μM) significantly decreased flow-induced dilatation in control but not in L-NAME or L-NAME + ACEI rats. Both acute indomethacin (10 μM) and acute NS 398 (cyclo-oxygenase-2 (COX-2) inhibitor, 1 μM) did not change significantly flow-induced dilatation in controls but they both decreased flow-induced dilatation in the L-NAME and L-NAME + ACEI groups. Acute Hoe 140 (bradykinin receptor inhibitor, 1 μM) induced a significant contraction of the isolated mesenteric arteries which was the same in the 3 groups.

6 Immunofluorescence analysis of COX-2 showed that the enzyme was expressed in resistance mesenteric arteries in L-NAME and L-NAME + ACEI groups but not in control. COX-1 expression was identical in all 3 groups.

7 We conclude that chronic inhibition of nitric oxide synthesis is associated with a decreased flow-induced dilatation in resistance mesenteric arteries which was compensated by an overproduction of vasodilator prostaglandins resulting in part from COX-2 expression. The decrease in flow-induced dilatation was prevented by the ACEI, quinapril.

Keywords: Myogenic tone; shear stress; resistance arteries; vascular reactivity; cyclo-oxygenase; L-NAME; angiotensin I converting enzyme inhibitors

Introduction

Nitric oxide (NO) is a labile vasodilator produced by vascular endothelial cells (Moncada *et al*., 1991; Moncada & Higgs, 1993). Flow-induced shear stress is probably the main stimulus for the continuous release of NO by the vascular endothelium and contributes to maintain a vasodilator tone (Kuo *et al*., 1992; Smiesko & Johnson, 1993; Bevan & Henrion, 1994). Diffuse NO blockade by N^G-nitro-L-arginine methyl ester (L-NAME) induces a substained hypertension in normotensive rats (Arnal *et al*., 1992; 1993; Baylis *et al*., 1992; Ribeiro *et al*., 1992). Long-term L-NAME administration represents a useful model of diffuse NO deficiency. In the aorta of L-NAME chronically treated rats there is a dramatic decrease in guanosine 3′:5′-cyclic monophosphate (cyclic GMP) content (Arnal *et al*., 1992) and a down-regulation of agonist-induced tone (Henrion *et al*., 1996). In small resistance mesenteric arteries we observed a decreased acetylcholine-induced dilatation and a decreased reactivity to vasoconstrictor agents which might represent a down-regulation of the contractile apparatus (Dowell *et al*., 1996). Finally, L-NAME-induced hypertension is prevented by angiotensin converting enzyme inhibitors (ACEI) and angiotensin II (AII) receptor (AT_1) antagonists (Sigmon & Beierwaltes, 1993; Brunner *et al*., 1993; Pollock *et al*., 1993; Qiu *et al*., 1994; Zanchi *et al*., 1995). ACEI have been shown to improve endothelium relaxant function in hypertension and arteriosclerosis (Holtz & Goetz, 1994). In the present study we investigated the vascular reactivity of small mesenteric resistance arteries to pressure and flow after chronic L-NAME administration.

Pressure-induced tone (myogenic tone) is a characteristic of small resistance arteries and of some veins (Bevan & Laher, 1991; Johnson, 1991; D'Angelo & Meininger, 1994). It is opposed by flow-induced dilatation, *in vitro* as well as *in vivo* (Bevan & Laher, 1991; Kuo *et al*., 1991; Smiesko & Johnson,

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1993; Bevan & Henrion, 1994; Segal, 1994). These two mechanical stimuli allow a constant basal tone to exist in resistance arteries and a rapid adaptation to changes in flow and pressure (Bevan & Laher, 1991; Smiesko & Johnson, 1993). Myogenic tone develops upon stretch of the vascular wall. It depends upon the activation of phospholipase C and protein kinase C (Laher & Bevan, 1989; Osol, 1991) and does not require a high level of intracellular calcium (Henrion *et al*., 1992). Myogenic tone is mainly independent of endothelial factors (D'Angelo & Meininger, 1994) and is increased in hypertension (Falcone *et al*., 1993). On the other hand, flow produces shear stress and triggers a dilatation (Bevan & Laher, 1991; Smiesko & Johnson, 1993; Segal, 1994). Flow-induced dilatation is in part dependent on the production of NO (Kuo *et al*., 1992; Hecker *et al*., 1993; Bevan & Henrion, 1994; Segal, 1994) and cyclo-oxygenase (COX) products (Hecker *et al*., 1993; Koller *et al*., 1993; Koller & Huang, 1994) by the endothelium. In the present study we tested the hypothesis that an attenuation in flow-induced dilatation might be involved in L-NAME-induced hypertension. Such an alteration in flow-induced dilatation would increase myogenic tone in resistance arteries. We also tested the hypothesis that suppression of the NO-dependent vasodilator tone would trigger compensatory mechanisms such as secretion of COX products. As ACEI prevent L-NAME-induced hypertension we studied the effect of treatment with L-NAME plus ACEI on flow-induced dilatation. Thus, myogenic tone and flow-induced dilatation were studied in mesenteric resistance artery segments isolated from rats treated for 3 weeks with L-NAME or L-NAME plus the ACEI, quinapril. In addition, the acute effect of NO synthase and COX inhibition on flow-induced dilatation was tested *in vitro*. We also performed an immunofluorescence analysis of COX-1 and COX-2 in the wall of the mesenteric resistance arteries.

Methods

L-NAME treatment and blood pressure measurement

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 approximately 50 mg kg^{-1} (Arnal *et al*., 1993). Other rats were given L-NAME (50 mg kg^{-1} day^{-1}) plus quinapril (10 mg kg^{-1} day^{-1}) in their drinking water for three weeks. In the control group rats were given tap water. After 3 weeks, rats were anaesthetized (pentobarbitone 50 mg kg^{-1}, i.v.) and the right carotid artery was cannulated (ID 0.6 mm) in order to measure blood pressure. The cannulae were connected to a pressure transducer (Gould P10EZ, spectramed, Oxnard, CA) and the signal was displayed on a chart recorder (Gould, Recording Systems Division, Cleveland, OH). 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 (Ministère de l'agriculture, France, authorization nb 00577).

Urinary measurement of dinor-6-keto-prostaglandin $F_{1\alpha}$ and dinor-thromboxane B_2

In a separate series of experiments, dinor-6-keto-prostaglandin $F_{1\alpha}$, a stable catabolite of prostacyclin of vascular origin (Lellouche *et al*., 1990), and dinor-thromboxane B_2, a stable catabolite of thromboxane A_2 which is produced selectively by COX-1 in platelets (FitzGerald *et al*., 1983) were measured in urine. After 3 weeks of treatment with L-NAME (50 mg kg^{-1} day^{-1} for 3 weeks) or L-NAME (50 mg kg^{-1} day^{-1}) plus quinapril (10 mg kg^{-1} day^{-1}) for 3 weeks, rats were placed in individual metabolic cages for a 24 h urine collection. In another series of experiments, rats were treated with L-NAME (50 mg kg^{-1} day^{-1}) as described above for 3 weeks and then received, in addition to L-NAME (50 mg kg^{-1} day^{-1}) the non-selective COX inhibitor flurbiprofen (5 mg kg^{-1} day^{-1}) or the COX-2 inhibitor NS 398 (5 mg kg^{-1} day^{-1}) for five days.

Urine volumes and creatinine were determined and urine samples frozen at $-80°C$. Purification and extraction of urine for enzyme immunoassay analysis was identical to that performed by Lellouche *et al*. (1990). Urinary creatinine was measured by the method of Jaffe (creatinine Combination-test, Boehringer Mannhein, Germany). Results were expressed as ng $mmol^{-1}$ creatinine.

Rat mesenteric artery

After the blood pressure measurement, as described above, heparin 1000 iu kg^{-1} was injected through the cannulae. Rat mesenteric artery segments, approximately 100 μm in internal diameter at 25 mmHg, were isolated, cannulated at both ends and mounted in a video monitored perfusion system (Halpern *et al*., 1984). The artery was bathed in a 5 ml organ bath containing physiological salt solution of the following composition (in mM): NaCl 135.0, $NaHCO_3$ 15.0, KCl 4.6, $CaCl_2$ 1.5, $MgSO_4$ 1.2, glucose 11.0, and N-2-hydroxy-ethylpiperazine-N-2-ethylsulphonic acid 5. The pH was 7.4. The artery was superfused at a rate of 4 ml min^{-1}. Perfusion of the artery with a physiological salt solution of the same composition was set as a rate of 100 μl min^{-1}. The pressure in the proximal end of the artery segment was monitored by a pressure transducer and controlled by a servoperfusion system. Arterial diameter was recorded by use of a video monitoring system (Living System Instrumentation Inc., Burlington, VT). Pressure and flow rate could be changed independently. Equilibrium diameter changes were measured in each segment when intraluminal pressure was set at 25, 50, 75, 100, 125 and 150 mmHg. Flow rate in the artery was either 0 or 100 μl min^{-1}. In another group of experiments pressure was set at 100 mmHg and flow was increased by steps from 0 to 100 μl min^{-1}. Arteries were submitted to pressure steps with or without intraluminal flow and this was subsequently repeated after addition of either N^G-nitro-L-arginine (L-NOARG, 10 μM), indomethacin (10 μM), NS 398 (1 μM) or Hoe 140 (1 μM) to the perfusate and superfusate. NS 398 is a selective COX-2 inhibitor (Futaki *et al*., 1994) and Hoe 140 is a specific bradykinin B_2 receptor blocker (Feletou *et al*., 1994). At the end of each experiment arteries were perfused and superfused with a Ca^{2+}-free physiological salt solution containing ethylenbis-(oxyethylenenitrolo) tetra-acetic acid (EGTA, 2 mM) and sodium nitroprusside (10 μM) and the pressure steps (25 to 150 mmHg) were repeated in order to determine the passive diameter (PD) of the vessel, i.e., in the absence of smooth muscle tone. Diameters measured in normal physiological salt solution were considered as diameter under active tone or 'active diameter' (AD) (Osol, 1991; Falcone *et al*., 1993; Davies & Tripathi, 1993). Pressure and diameter measurements were collected by a Biopac data aquisition system (Biopac MP 100, La Jolla, CA, U.S.A.), recorded and analysed on a Macintosh Quadra computer (Apple, Cupertino, CA, U.S.A.) by the Acknowledge software (Biopac, La Jolla, CA, U.S.A.). Results are given in μm for arterial diameters. Myogenic tone was expressed as percentage of passive diameter (measured diameter/passive diameter $\times$ 100. Flow-induced dilatation was expressed as percentage of relaxation of myogenic tone (difference between myogenic tone with and without flow/myogenic tone without flow $\times$ 100) or as increases in diameter induced by flow (μm).

Immunofluorescence analysis of COX-1, COX-2

Approximately 2 mm long segments of a mesenteric resistance artery were collected in control, L-NAME and L-NAME plus quinapril treated rats in order to perform an immunostaining analysis. Segments were mounted in embedding medium (Miles, Inc., Elkhart, U.S.A.) and frozen in isopentane previously cooled in liquid nitrogen. Frozen segments were stored at $-80°C$. Transverse cross section (5 μm thick) were obtained

at −20°C from the frozen embedded segments. Sections were incubated overnight at 4°C with specific anti-COX-1 or anti-COX-2 antibodies and then incubated for 30 min at 37°C with anti-rabbit antibodies conjugated to biotin (Amersham Int plc, U.K.) which was amplified by streptavidin-Texas-red reagent (Amersham Int plc, U.K.). Fluorescence staining was visualized by a microscope equipped with an epifluorescence system (Leica, Rueil-Malmaison, France). Rabbit polyclonal anti-rat COX-1 antibodies were used at a dilution of 1 : 200 and rabbit polyclonal anti-rat COX-2 antibodies were used at a dilution of 1 : 800. These antibodies were provided by Dr J. Maclouf and have been shown to be specific (Rimarachin *et al*., 1994; Créminon *et al*., 1995). Control experiments were performed to verify the specificity of the antibodies in which the second antibody (anti-specific-specific antibody) only was present or in which the first antibody (anti-COX-1 or COX-2-specific antibody) only was present. In these 2 series of controls no self-fluorescence was observed.

Statistical analysis

Results are expressed as means ± s.e.mean. Significance of the differences between the different groups were determined by analysis of variance (one factor ANOVA, or two factor ANOVA for consecutive measurements, as appropriate). Means were compared by Dunnett's or Bonferroni's test. P values less than 0.05 were considered to be significant.

Drugs

N-2-hydroxy-ethylpiperazine-N-2-ethylsulphonic acid, N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-L-arginine (L-NOARG), indomethacin, flurbiprofen and ethylenbis-(oxyethylenenitrolo) tetra-acetic acid (EGTA) were purchased from Sigma (St Louis, MO). NS 398 (*N*-(2-cyclohexyloxy)-4-nitrophenyl)methanesulphonamide) was purchased from SPIBIO (Massy, France). Quinapril was given by Parke Davis-France and Hoe 140 (D-Arg-Arg-Pro-Hyp-Gly-Thr-Ser-D-Tic-Oic-Arg acetate) by Hoechst-France. Other reagents were purchased from Prolabo (Paris, France).

Results

Blood pressure and survival rate

Chronic L-NAME treatment induced a significant decrease in the gain of body weight as compared with control (309 ± 7 g, $n=8$, vs 342 ± 6 g, $n=10$; $P<0.05$) which was only partly restored by quinapril (326 ± 6 g, $n=14$). Mean arterial pressure, measured in the carotid artery, was significantly increased by chronic L-NAME (155 ± 4 mmHg, $n=8$, vs control 121 ± 6 mmHg, $n=10$; $P<0.05$). Chronic L-NAME-induced hypertension was prevented by quinapril (130 ± 4 mmHg, $n=14$).

The non-selective COX-1 and -2 inhibitor flurbiprofen (5 mg kg^{-1}) and the selective COX-2 inhibitor NS 398 (5 mg kg^{-1}) had no significant effect on systolic blood pressure measured by the tail cuff method. Systolic blood pressure was 190 ± 4 mmHg ($n=5$) in chronic L-NAME-treated rats, 194 ± 20 mmHg in chronic L-NAME + flurbiprofen-treated rats ($n=5$) and 197 ± 7 mmHg in chronic L-NAME + NS 398-treated rats ($n=5$).

Urinary dinor-6-keto-prostaglandin $F_{1\alpha}$ and dinor-thromboxane B_2

Dinor-6-keto-prostaglandin $F_{1\alpha}$ was significantly increased in the urine of chronic L-NAME-treated rats compared to control animals. In the group treated chronically with L-NAME and quinapril urinary dinor-6-keto-prostaglandin $F_{1\alpha}$ was not significantly different from control (Figure 1). In L-NAME-treated rats the selective COX-2 inhibitor NS 398 (5 mg kg^{-1}) and the non selective COX inhibitor flurbiprofen (5 mg kg^{-1}) significantly decreased urinary dinor-6-keto-prostaglandin $F_{1\alpha}$ Flurbiprofen (5 mg kg^{-1}) induced a significantly larger decrease in urinary dinor-6-keto-prostaglandin $F_{1\alpha}$ than NS 398. In control rats, flurbiprofen induced a decrease in urinary dinor-6-keto-prostaglandin $F_{1\alpha}$ similar to that observed in L-NAME-treated rats, whereas NS 398 had no significant effect (not shown).

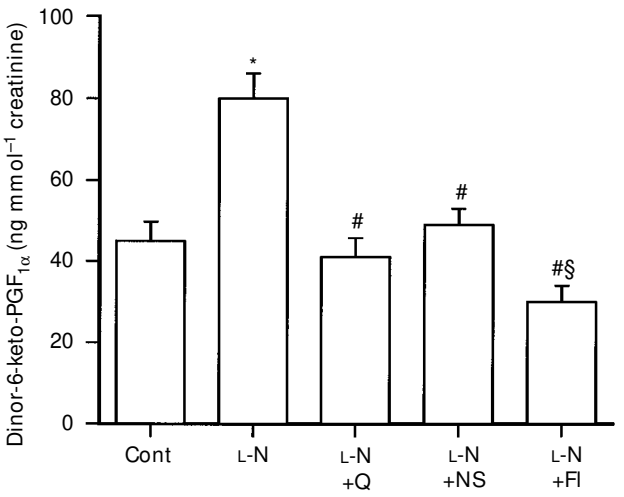

Figure 1 Dinor-6-keto-prostaglandin $F_{1\alpha}$ measured in the urine of rats treated chronically with L-NAME (L-N, $n=14$) or L-NAME + quinapril (L-N + Q, $n=9$) as compare to control (Cont, $n=8$). In another series of experiments the COX-1 and COX-2 inhibitor flurbiprofen (5 mg kg^{-1}, L-N + Fl, $n=5$) or the COX-2 inhibitor NS 398 (5 mg kg^{-1}, L-N + NS, $n=5$) were given to L-NAME-treated rats for 5 days. Data are expressed as ng mmol^{-1} creatinine (mean ± s.e.mean). *$P<0.05$, significantly different from the control group. #$P<0.05$, significantly different from the L-NAME treated group. §$P<0.05$, significantly different from the L-NAME + NS 398 group.

In order to evaluate prostaglandin production derived solely from COX-1, we measured dinor-thromboxane B_2, the urinary metabolite of thromboxane A_2 from platelets (FitzGerald *et al*., 1983). In L-NAME-treated rats NS 398 (5 mg kg^{-1}) had no significant effect on urinary dinor-thromboxane B_2 (74 ± 7 vs 78 ± 8 ng mmol^{-1} creatinine, $n=5$ per group). On the other hand, in L-NAME-treated rats, flurbiprofen (5 mg kg^{-1}) significantly decreased urinary dinor-thromboxane B_2 (12 ± 3 vs 78 ± 8 ng mmol^{-1} creatinine, $n=5$ per group). Urinary dinor-thromboxane B_2 was not affected by chronic L-NAME and both flurbiprofen (5 mg kg^{-1}) and NS 398 (5 mg kg^{-1}) had the same effect in control and L-NAME-treated rats (data not shown).

Isolated mesenteric resistance arteries

Step increases in intraluminal pressure induced the development of myogenic tone (Figure 2). Internal diameter was higher in the presence of flow, reflecting a flow-induced dilation (Figure 3). Passive diameter values obtained in a Ca^{2+}-free physiological salt solution containing EGTA (2 mM) and sodium nitroprusside (10 μM) in L-NAME and L-NAME + quinapril groups were not significantly different from those in control conditions (Figure 2). In the L-NAME and L-NAME + quinapril groups myogenic tone was not significantly different from control (Figure 2). The addition of L-NOARG (10 μM), indomethacin (10 μM), NS 398 (1 μM) or Hoe 140 (1 μM) to the perfusate and to the superfusate induced no significant change in myogenic tone in all three groups (data not shown).

Flow-induced relaxation was significantly reduced in the L-NAME group as compared to control (Figure 3), There was no significant difference in flow-induced dilatation between L-NAME + quinapril and control groups (Figure 3). The addi-

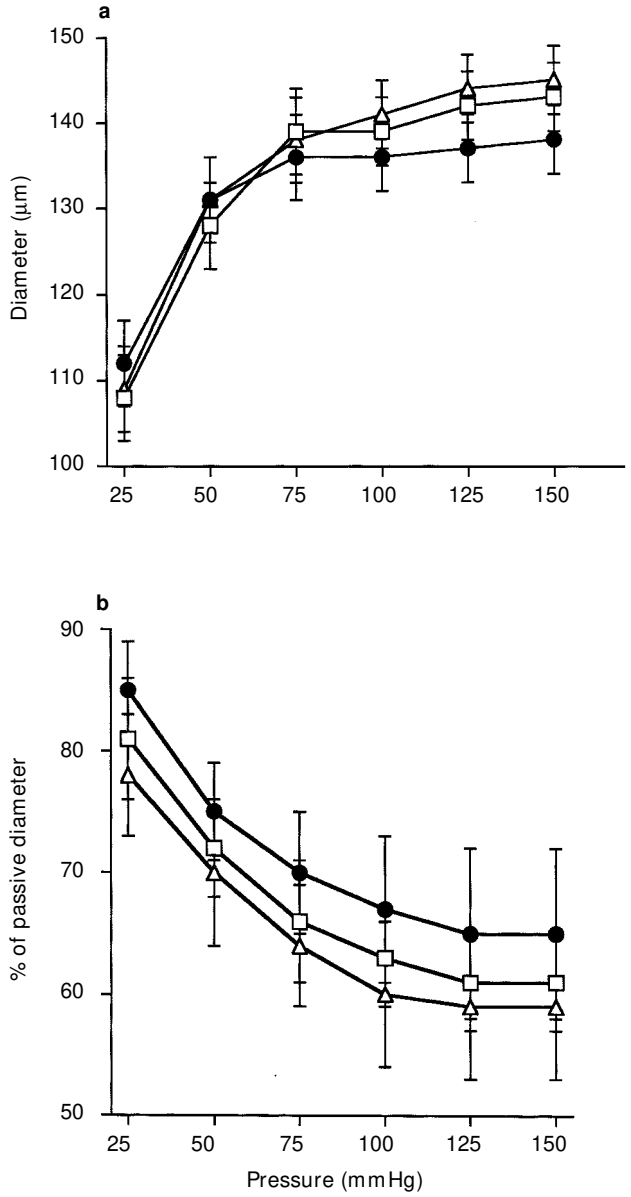

Figure 2 Myogenic tone (b) and passive diameter (a) determined in mesenteric resistance artery segments isolated from rats treated for 3 weeks with L-NAME (●) or L-NAME + quinapril (△) and from control rats (□). Myogenic tone was expressed as % of passive diameter (b). Passive diameter was determined in artery segments bathed in a Ca^{2+}-free physiological salt solution containing EGTA (2 mM) and sodium nitroprusside (10 μM). Data are expressed as mean, $n = 8$ to 14 per group; vertical lines show s.e.mean. Two factors analysis of variance (ANOVA) for consecutive measurements were made and no significant difference between groups was found.

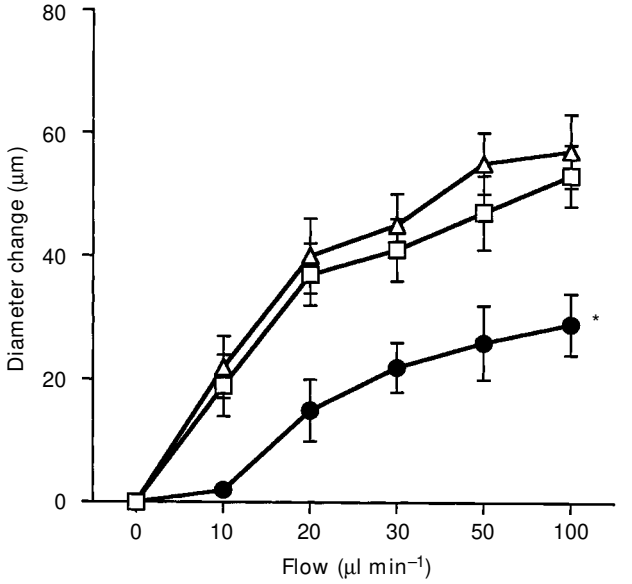

Figure 3 Flow-induced dilatation in mesenteric artery segments isolated from rats treated for 3 weeks with L-NAME (●) or L-NAME + quinapril (△) and from control rats (□). Pressure was set at 100 mmHg and flow steps were performed from 0 to 100 μl min^{-1}. Data are expressed as mean of changes in diameter (μm), $n = 8$ to 14 per group; vertical lines show s.e.mean. $^*P = 0.0001$; two factors analysis of variance (ANOVA) for consecutive measurements, compared to control.

tion of L-NOARG (10 μM) to the perfusate and to the superfusate significantly decreased flow-induced relaxation in the control group but was without effect in the L-NAME and L-NAME + quinapril groups (Figure 4). The addition of indomethacin (10 μM) induced no further significant change in flow-induced relaxation in the control group. On the other hand, the addition of indomethacin (10 μM) further decreased flow-induced relaxation in the L-NAME and L-NAME + quinapril groups (Figure 4). NS 398 (1 μM) induced a significant attenuation of flow-induced dilatation in the L-NAME ($P < 0.01$, $n = 7$) and L-NAME + quinapril groups ($P < 0.005$, $n = 7$) without affecting significantly flow-induced dilatation in the control group (Figure 4). NS 398 (1 μM) was significantly less effective at attenuating flow-induced dilatation than indomethacin (10 μM) (Figure 4). NS 398 (0.1 μM) had no significant effect on flow-induced dilatation, whereas the effect of NS 398 (10 μM) was not significantly different from that due to NS 398 (1 μM) (not shown). The bradykinin B_2 receptor blocker Hoe 140 (0.1 and 1 μM) had no significant effect on flow-induced dilatation (data not shown).

Immunostaining of COX-1 and COX-2

COX-1 immunostaining in the mesenteric resistance arteries showed that the enzyme is located in the endothelium of the mesenteric arteries of both L-NAME-treated and control rats, without significant differences between groups (Figure 5). COX-1 immunostaining in the mesenteric resistance arteries in L-NAME + quinapril-treated rats was not significantly different from control.

Immunostaining of COX-2 was significant in mesenteric arteries from the L-NAME (Figure 5) and L-NAME plus quinapril (not shown) treated rats, whereas no significant staining was found in the control gorup (Figure 5). Immunostaining of COX-2 was located in the tunica media and the endothelium of the mesenteric resistance arteries (Figure 5).

Discussion

Chronic administration of L-NAME induced a sustained hypertension which was prevented by the ACEI, quinapril. This hypertension has been attributed to a decrease in the continuous production of NO by vascular endothelial cells (Moncada *et al.*, 1991; Moncada & Higgs, 1993). The model could be described as a two stage model. In the early stage the plasma renin activity tends to be lower than in control (Arnal *et al.*, 1992) and in the second stage, beginning after 2 months, activation of the renin-angiotensin system appears in relation to arteriolar damage in the kidney (Xu *et al.*, 1995). Despite the absence of renin and angiotensin overproduction, angiotensin II receptor blockers (ATI), such as losartan (Brunner *et al.*, 1993; Pollock *et al.*, 1993; Sigmon *et al.*, 1993; Qiu *et al.*, 1994; Zanchi *et al.*, 1995), and α-adrenoceptor blockers, such as

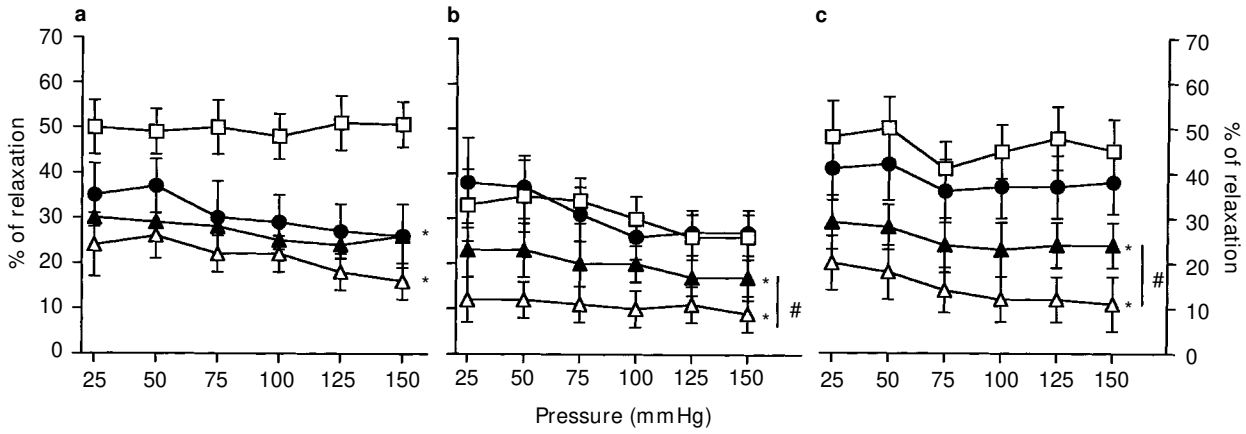

Figure 4 Flow-induced dilatation in mesenteric artery segments isolated from (a) control rats and from rats treated for 3 weeks with (b) L-NAME or (c) L-NAME + quinapril. Each group of artery segments was treated (intra and extraluminaly) with L-NOARG (10 μM, ●), indomethacin (10 μM, △) or NS 398 (1 μM, ▲) for 30 min. In the control group (□) artery segments were treated with the solvent for indomethacin (ethanol 0.1%). L-NOARG (10 μM) was dissolved in physiological salt solution. Flow-induced dilatation was estimated as the difference between myogenic tone with and without flow expressed as percentage of relaxation. Data are expressed as mean, $n=8$ to 14 per group; vertical lines show s.e.mean. $^*P<0.01$; two factors analysis of variance (ANOVA) for consecutive measurements, compared to control. $\#P<0.05$; two factors analysis of variance (ANOVA) for consecutive measurements, indomethacin compared to NS 398.

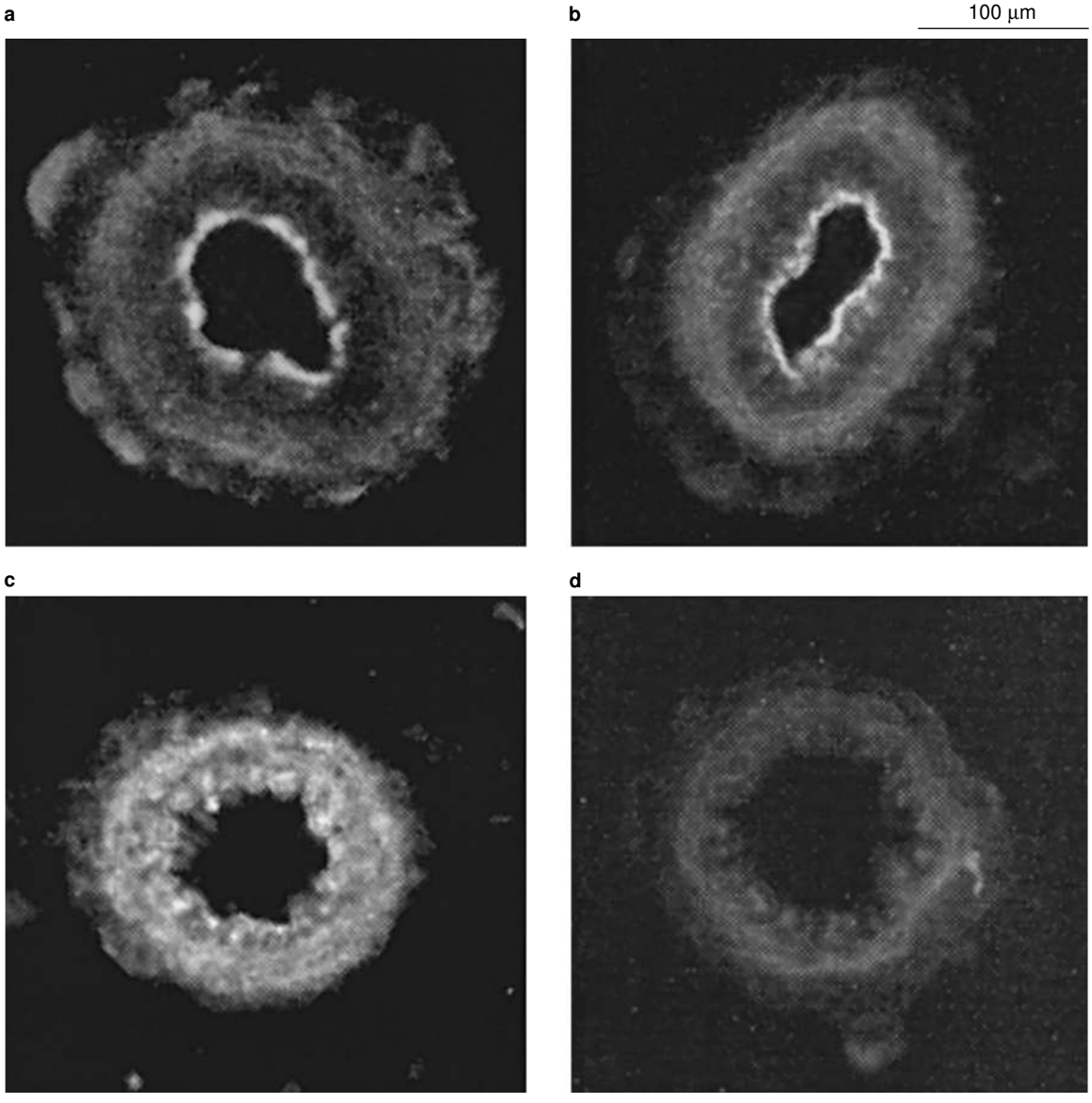

Figure 5 Immunostaining of cyclo-oxygenase-1 (a, b) and cyclo-oxygenase-2 (c, d) in mesenteric resistance artery segments isolated from rats treated for 3 weeks with L-NAME (a, c) and from control rats (b, d). Each view shown is representative of 8 to 10.

prazosin (Qui *et al*., 1994) and phentolamine (Zanchi *et al*., 1995), reduce L-NAME-induced hypertension. In our experiments, flow-induced dilatation was attenuated by chronic L-NAME administration. This is consistent with previous observations demonstrating a role for NO in flow-dependent responses (Kuo *et al*., 1992; Smiesko & Johnson, 1993; Bevan & Henrion, 1994) and also with the experiments in our control group in which *in vitro* acute L-NOARG decreased flow-induced dilatation by approximately 50%. That neither chronic nor acute NO synthesis blockade could completely suppress flow-induced dilatation is consistent with the observations that shear stress induces not only the release of NO but also triggers the production of prostaglandins (Hecker *et al*., 1993; Koller *et al*., 1993; Koller & Huang, 1994), hyperpolarizing factor (Furchgott & Vanhoutte, 1991) and probably other factors (Davies & Tripathi, 1993; Bevan & Henrion, 1994). In L-NAME-treated rats indomethacin attenuated flow-induced dilatation whereas no significant effect was observed in the control group. Thus in chronic L-NAME-treated rats a compensatory increase in the production of relaxant prostaglandins in response to flow occurred. This increase in prostaglandin production in response to flow probably involved both COX-1 and COX-2 in mesenteric resistance arteries from L-NAME rats, as suggested by our immunostaining results and by our observation that in L-NAME-treated rats both the COX-2 inhibitor NS 398 and indomethacin decreased flow-induced dilatation. That indomethacin decreased flow-induced dilatation significantly more than NS 398 suggest that both COX-1 and COX-2 produced prostaglandins. This was confirmed by our finding that urinary dinor-6-keto-prostaglandin $F_{1\alpha}$ was decreased by both flurbiprofen and NS 398, but significantly more by flurbiprofen. We also performed the measurement of the urinary metabolite dinor-thromboxane B_2 which was decreased only by flurbiprofen. As dinor-thromboxane B_2 is mainly produced by platelets which contrain only COX-1 (Habib *et al*., 1994), we can assume that COX-1 is not affected by chronic L-NAME, thus supporting our finding that increased COX-2 expression is located mainly in the vascular wall. The participation of prostaglandins in the response to flow has been shown in gracilis muscle small arteries (Koller & Huang, 1994), rat cremaster arterioles (Koller *et al*., 1993), in rabbit femoral arteries (Hecker *et al*., 1993) as well as in dog pulmonary arteries (Barnard *et al*., 1993). Regarding L-NAME-induced hypertension, this might represent a mechanism by which the decreased NO production would be partly compensated. Nevertheless vascular beds are different from each other and our observation in the mesenteric circulation might have to be considered carefully regarding other vascular territories. Moreover, it should be pointed out that the ACEI reduced substantially L-NAME-induced hypertension and the associated urinary secretion of dinor-6-keto-prostaglandin $F_{1\alpha}$, whereas flow-induced dilatation remained dependent upon prostaglandins in L-NAME + quinapril-treated rats. Thus, this compensatory production of prostaglandins might represent only part of the increased excretion of dinor-6-keto-prostaglandin $F_{1\alpha}$ in the urine of L-NAME-treated rats. Increased urinary secretion of dinor-6-keto-prostaglandin $F_{1\alpha}$ might be more representative of an increase in vascular PGI_2 secretion due to the increased blood pressure which produces more stretch on the vessels wall. Indeed, mechanical stimulation increases cyclo-oxygenase activity (Vandenburgh *et al*., 1995). The positive immunostaining for COX-2 in L-NAME-treated rats suggests that specific regulatory mechanisms may contribute to the expression of COX-2. Among the multiple mechanisms, suppression of NO synthase activity by L-NAME could constitute the biochemical basis for this regulation as various interactions exist between the two systems (Swierkosz *et al*., 1995). We found COX-1 in endothelial cells and COX-2 in smooth muscle and endothelial cells. This is consistent with previous studies which have shown the presence of the enzyme in the different cell types (Rimarachin *et al*., 1994; Créminon *et al*., 1995; Swierkosz *et al*., 1995). Indomethacin and NS 398 attenuated flow-induced dilatation in mesenteric resistance arteries from L-NAME and L-NAME + quinapril-treated rats.

The ACEI quinapril prevented the development of hypertension and the decrease in flow-induced dilatation due to L-NAME. ACEIs, which prevent the conversion of angiotensin I into angiotensin II, also act through the inhibition of bradykinin breakdown (Mombouli *et al*., 1992; Unger & Lebrun, 1992) and through other mechanisms such as α-adrenoceptor blockade (Vanhoutte *et al*., 1989). Bradykinin action through the activation of the L-arginine-NO pathway is blocked by L-NAME. Nevertheless, ACEIs elevate endothelial intracellular calcium concentration (Busse & Lamontagne, 1991), probably by inhibiting the breakdown of bradykinin which in turn stimulates calcium entry in endothelial cells and thus calcium can stimulate phospholipase A_2 and prostaglandins production (Wiemer *et al*., 1991; 1994). Nevertheless, bradykinin receptor blockade with Hoe 140 had no effect on flow-induced dilatation in either group. Thus the mechanism by which ACEIs act on flow-induced dilatation in L-NAME-treated rats remains to be determined. Such a compensatory mechanism involving prostaglandins has been described in resistance arteries of hypertensive rats (Koller & Huang, 1994). We found that dinor-6-keto-prostaglandin $F_{1\alpha}$ was increased in the urine of L-NAME-treated rats but not in the urine of rats treated with L-NAME + quinapril. This result might look paradoxical as flow-induced dilatation depends upon prostaglandin production in both L-NAME and L-NAME + quinapril rats, quinapril normalizes blood pressure and ACEIs have been described as increasing prostaglandin synthesis (Wiemer *et al*., 1991; 1994). There are several possible explanations to this paradox. COX production involved in flow-induced dilatation in L-NAME-treated rats might represent only a small proportion of the PGI_2 secretion, or other dilator prostaglandins might be involved in flow-induced dilatation in mesenteric resistance arteries from L-NAME-treated rats. Urinary dinor-6-keto-prostaglandin $F_{1\alpha}$ might also represent a production of PGI_2 due to pressure, as discussed above. An alternative possibility would be that the increased vascular prostacylin production would be triggered by angiotensin II, as angiotensin II activates prostaglandin synthesis (Pfeilschifter & Bauer, 1986; Ford & Gross, 1989). ACEI which decreased blood pressure in L-NAME-treated rats would thus normalize the level of dinor-6-keto-prostaglandin $F_{1\alpha}$ found in the urine of L-NAME plus quinapril treated rats. But this latter possibility might not apply in the present study as ACEIs increase prostaglandin production by decreasing the breakdown of bradykinin (Wiemer *et al*., 1991; 1994) and we found that the bradykinin B_2 receptor blocker had no effect on flow-induced dilatation in our study. Thus, it seems that the balance between activator and inhibitor mechanisms of COX is modified in L-NAME-treated rats and no definitive conclusion can be drawn concerning the exact role of prostaglandins in L-NAME-induced hypertension, excepted that COX products, instead of nitric oxide, mediate flow-induced dilatation in resistance mesenteric arteries from L-NAME-treated rats.

Myogenic tone in the absence of flow was not significantly modified by L-NAME or L-NAME + quinapril. This is in agreement with previous studies showing that the endothelium is of little importance in pressure-induced tone in small resistance arteries (see D'Angelo & Meininger, 1994 for review).

In conclusion, chronic L-NAME-treatment induced a decrease in flow-induced dilatation in mesenteric resistance arteries and a loss of NO-dependent dilatation. This loss in flow-induced production of NO was compensated by the production of prostaglandins by both COX-1 and COX-2.

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