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Comparison of the nitric oxide and cyclo-oxygenase pathway in mesenteric resistance vessels of normotensive and spontaneously hypertensive rats

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1 The double perfused mesentery was used to compare arterial and venous KCl- and acetylcholine (ACh)-induced responses in tissues taken from normotensive (WKY) and spontaneously hypertensive rats (SHR), in the presence or absence of inhibitors of nitric oxide (NO) synthase (N^G-nitro-L-arginine (L-NOARG) and N^G-nitro-L-arginine methyl ester (L-NAME)) and cyclo-oxygenase (indomethacin, mefenamic acid).

2 KCl (20 to 120 mM K^+) caused concentration-dependent increases in arterial and venous perfusion pressures. The maximal arterial effects were significantly higher in the SHRs than in the WKY, with no differences in the venous pressor responses.

3 L-NAME and L-NOARG (100 μM) had no effect on the basal perfusion pressures in tissues from either WKY or SHRs, and mefenamic acid only induced a significant reduction of the basal perfusion pressures in the venous mesenteric vessels isolated from WKY.

4 L-NAME and L-NOARG (100 μM) potentiated the pressor responses to KCl to the same extent in the venous and arterial beds derived from WKY and SHR, while indomethacin and mefenamic acid (5 μM) only significantly decreased these responses in WKY.

5 Acetylcholine (ACh)-induced relaxations (1 nM to 10 μM) were significantly higher in arterial beds of WKY than in SHR, without differences in the venous relaxant responses.

6 L-NAME (100 μM) inhibited ACh-induced relaxations in arterial and venous beds from both groups of rats. Mefenamic acid was without effect on ACh-induced relaxations in either the arterial or the venous beds from WKY and SHR.

7 In conclusion, the liberation of NO in the perfused mesenteric vasculatures requires an active tone and no dysfunction of NO synthase activity is functionally apparent in the mesenteries isolated from SHRs. The cyclo-oxygenase pathway is only implicated in the KCl-induced responses of tissues derived from WKY, but not in the vasodilatations induced by ACh in either the arterial or the venous vasculatures from WKY and SHR.

Keywords: Nitric oxide; cyclo-oxygenase pathway; mesenteric resistance vessels; spontaneously hypertensive rats; NO synthase inhibitors; acetylcholine; extracellular K^+

Introduction

A variety of vasodilator agents such as acetylcholine (ACh) require an intact endothelium to relax the vascular smooth muscle, secondary to the liberation of the endothelium-derived relaxing factor (EDRF), or nitric-oxide (NO; Palmer *et al.*, 1987). ACh has also been shown to cause smooth muscle hyperpolarization, secondary to the liberation of the endothelium-derived hyperpolarizing factor (EDHF; Félétou & Vanhoutte, 1988). However, there are conflicting data as to the relative contributions of NO and EDHF as mediators of endothelium-dependent relaxation in response to ACh in the perfused mesenteric bed isolated from normotensive rat. Indeed, N^G-nitro-L-arginine (L-NOARG) was found to abolish ACh-evoked dilatation by Moore *et al.* (1990), while Parsons *et al.* (1994) noted that ACh-evoked responses were resistant to the blocking action of NO synthase inhibitors. Considerable evidence now shows that, in fact, ACh-evoked vasodilatation of the rat mesenteric arterial vasculature is mediated by both NO-dependent and -independent mechanisms. For instance endothelium-dependent vasodilator responses are markedly inhibited by K^+ channel antagonists, suggesting that acetylcholine-evoked vasodilatations of the rat mesenteric vasculature are mediated by an EDHF, which initiates vasodilatation through activation of an apamin-sensitive K^+ channel (Adeagbo & Triggle, 1993). Moreover, the observation that indomethacin does not reduce the hyperpolarization caused by ACh (Félétou & Vanhoutte, 1988; Garland & McPherson, 1992) indicates that EDHF is not a product of cyclo-oxygenase activity. While in large arteries EDHF may provide only a complementary system to NO, in small resistance arteries it appears to be a major determinant of the regulation of vascular resistance, since in these vessels endothelium-dependent vasodilator responses are markedly inhibited by K^+ channel antagonists.

The cardiovascular system of the spontaneously hypertensive rat (SHR) is characterized by functional and structural alterations compared to normotensive animals. In mesenteric resistance arteries, which are involved in the control of blood pressure, vascular changes are responsible for functional alterations such as increases in contractile responses. While it is now well established that a decreased responsiveness to endothelium-dependent vasodilator substances is characteristically seen in vessels taken from various models of experimental hypertension (Lüscher & Vanhoutte, 1986), there are conflicting data about the status of endothelial function in resistance vessels during hypertension. For example, Dohi *et al.* (1990) observed an impairment of endothelial function in the mesenteric vascular bed during hypertension, while Randall *et al.* (1991) in the same isolated preparation did not find such an impairment.

Relaxations induced by the endothelium-independent vasodilator sodium nitroprusside are not impaired in small ar-

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teries from hypertensive rats, suggesting there is a functional impairment of the endothelium rather than an altered vascular responsiveness to NO in this pathology. Moreover, the impaired endothelium-dependent relaxations to acetylcholine found in mesenteric resistance vessels of SHRs were improved following treatment with indomethacin (a cyclo-oxygenase inhibitor), suggesting release of a constrictor prostanoid (Lüscher *et al*., 1990) which underlies the increased vascular tone seen.

Alterations of venous function have been suggested to occur in experimental hypertension. For example, Greenberg & Bohr (1975) demonstrated a decreased passive extensibility *in vitro* of portal veins taken from spontaneously hypertensive rats. It is of interest, therefore, to compare the arterial and venous responses evoked by constrictor or relaxant agents. Here, we have studied, the double perfused venous and arterial mesenteric vascular bed, (Warner, 1990) in order to compare directly arterial and venous responses.

The aim of this study was to investigate the relative importance of NO-dependent and -independent mechanisms in mediating endothelium-dependent dilatations induced by ACh, in the perfused arterial and venous vasculatures isolated from Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). As increases in extracellular $[K^+]$ reduce the $[K^+]$ gradient between the outside and inside of the cell and decrease the effect of EDHF, we measured the relaxant effect of ACh in presence of an increased K^+-concentration and in the presence of inhibitors of nitric oxide synthase (NOS) and cyclo-oxygenase (COX).

Methods

Double perfused mesentery preparations

We have used genetically hypertensive animal models, the spontaneously hypertensive rats (SHR), and their normotensive controls, Wistar-Kyoto (WKY/NIco). Male rats (WKY and SHR) (250–300 g) were obtained from Iffa Credo France. Systolic blood pressure was measured in conscious rats by the tail-cuff method, to confirm the presence or the absence of hypertension in both groups.

The rat mesentery was prepared as previously described (Warner, 1990). Briefly, the rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.), and heparinized (500 iu, i.v.). The abdomen was then opened and the mesenteric artery and vein were quickly cannulated (Biotrol, internal diameter: 0.86 mm) at their origins. The mesentery was separated from the intestine by cutting close to the intestinal border. The venous and arterial vascular beds were subsequently perfused independently at a rate of 2 ml min^{-1}, with warmed (37°C) and gassed (95% O_2 and 5% CO_2) Krebs solution. Arterial and venous perfusion pressures were detected with P23XL transducers (Gould), and displayed continuously on a Linseis (L6514) recorder.

Experimental protocols

Series 1: KCl-induced responses After an initial equilibration period of 60 min, the arterial and venous mesenteric vasculatures of both WKY and SHR were precontracted for 10 min with a submaximal concentration of 100 mM of KCl. Following washout, and a second equilibration period of 30 min, raised K^+-Krebs solutions (20 to 120 mM KCl with a concomitant reduction of NaCl) were infused (15 min) to establish concentration-response curves to KCl; 15 min was sufficient for responses to reach a plateau.

The same experiments were performed to determine the effects of two inhibitors of NO synthesis, N^G-nitro-L-arginine (L-NOARG), N^G-nitro-L-arginine methylester (L-NAME), and two inhibitors of cyclo-oxygenase, indomethacin and mefenamic acid, on the concentration-response curves to KCl. Concentrations of 100 μM were used for the NOS inhibitors and 5 μM for the COX inhibitors, these have been shown to be effective concentrations to inhibit NOS (Rees *et al*., 1990) and COX (Yang *et al*., 1991). The NOS and COX inhibitors were added to the Krebs reservoir and allowed to perfuse the arterial and venous beds starting 20 min before and continuing during the infusion of KCl. In another series of experiments the endothelial layer of the mesenteries was removed by brief perfusion of CHAPS (20 mM, 30 s), before concentration-response curves to KCl were established.

Series 2: ACh-induced responses After an initial equilibration period of 60 min, the arterial and venous mesenteric vasculatures derived from WKY and SHR were precontracted for 10 min with a submaximal concentration of KCl (100 mM). Following washout and a second equilibration period of 30 min, the mesenteric vasculatures were precontracted by infusion of raised K^+-Krebs solution (100 mM KCl) and infusions of ACh (1 nM to 10 μM) given to establish the concentration-response curves. Infusion of ACh for 15 min was sufficient to obtain equilibrated pressures.

To determine the effects of the inhibitor of NOS, L-NAME (100 μM) was perfused for 20 min in Krebs solution (5 mM), then arterial and venous tones were increased by an infusion of 40 mM KCl in combination with L-NAME, and the responses to ACh (1 nM and 10 μM) were established during the infusion of L-NAME.

Drugs

The Krebs buffer had the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.17, $NaHCO_3$ 25 and glucose 11. The following drugs were used: sodium pentobarbitone (Sanofi), heparin (Leo), indomethacin, mefenamic acid, trizma base, N-nitro-L-arginine (L-NOARG), N-nitro-L-arginine methyl ester (L-NAME) and 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulphonate (CHAPS), which were all purchased from Sigma Chemical Co. All drugs were dissolved in distilled water, except indomethacin which was dissolved in Trizma base (pH 7.4; 0.2 M). Raised K^+-Krebs solutions (20 to 120 mM KCl) were prepared by direct replacement of NaCl with KCl. The maximally depolarizing potassium physiological salt solution that it was possible to prepare was equal to 120 mM.

Statistical analysis

Results are shown as mean values $\pm$ s.e.mean for $n=6-8$ experiments. Differences between two means were determined by unpaired Student's t test. A P value of less than 0.05 was taken as significant.

Results

Blood pressure

Rat blood pressure from both groups was measured at the beginning of the study. Values were respectively in WKY and SHR: 121 ± 3 mmHg and 146 ± 4 mmHg ($P<0.001$, $n=12$).

Basal arterial (APP) and venous perfusion pressures (VPP): effect of NOS and COX inhibitors and CHAPS

In spite of significant differences in systolic pressures between the groups, there were no significant differences between the basal perfusion pressures of the arterial and venous vasculatures of WKY and SHR, when we used the same flow of 2 ml min^{-1} (arterial perfusion pressure, WKY 9.0 ± 0.8, SHR 10.8 ± 1.5 mmHg; venous perfusion pressure, WKY 0.8 ± 0.2, SHR 1.0 ± 0.1 mmHg; $n=12$ for each).

Infusions of NOS inhibitors (L-NAME, L-NOARG) did not alter the basal arterial or venous perfusion pressure in tissues from either group of rats. The COX inhibitors (in-

domethacin, mefenamic acid) caused small but not significant reductions of the basal arterial perfusion pressure in mesenteries isolated from SHRs, while mefenamic acid induced a significant reduction ($P<0.05$) of the venous perfusion pressure of mesenteries isolated from WKY rats but not from SHRs (Table 1).

Perfusion of CHAPS (20 mM, 30 s) induced a transient increase in APP and VPP. The maximum increases observed in APP and VPP were, respectively,: $+10\pm1$ mmHg and $+2\pm0.7$ mmHg; but 15 min after these changes, the arterial and venous basal perfusion pressures were restored.

KCl-induced responses of arterial and venous beds

KCl (20 to 120 mM K^+) caused concentration-dependent increases in arterial and venous perfusion pressures (Figure 1). The contractile response to KCl (100 and 120 mM) in the arterial mesenteric vasculature was significantly higher in the SHRs than in the WKY. For instance 120 mM KCl increased the perfusion pressures in mesenteric arterial beds from WKY and SHRs by $+144\pm25$ and $+200\pm7.3$ mmHg ($n=6$, $P<0.05$). No significant differences were observed at the lower KCl concentrations used. The venous pressor responses to KCl (20 to 120 mM) were not significantly different between tissues from the groups of rats. At the higher concentration tested (120 mM), the venous perfusion pressures in preparations from WKY and SHRs increased by $+7.6\pm0.8$ and $+6.5\pm1.1$ mmHg ($n=6$), respectively.

Effect of NOS inhibitors on KCl-induced responses

L-NAME (100 μM) and L-NOARG (100 μM) potentiated significantly the pressor responses to KCl in both the arterial and venous vessels from WKY and SHRs (Figure 2). In arterial beds, in the presence of L-NAME, a maximum effect was observed after perfusion of KCl 100 mM (WKY and SHR equal to: WKY, $+142\pm7.2$; SHR, $+190\pm10$ mmHg; $n=6$). In the venous beds L-NAME also potentiated the effects of KCl. L-NOARG caused similar potentiations with KCl responses as did L-NAME.

There was no apparent difference in the potentiation of the pressor effects induced by NOS inhibitors in the arterial and venous mesenteric beds between the two groups of rats. Thus, NOS inhibitors potentiated the pressor responses to the same extent in the venous and arterial beds derived from WKY and SHR.

Effect of COX inhibitors on KCl-induced responses

Indomethacin (5 μM) and mefenamic acid (5 μM) significantly decreased responses to KCl in the arterial and venous vasculatures derived from WKY but not those from SHR (Figure 3). For example, in mesenteric arterial beds isolated from WKY, the pressure increases induced by perfusion of KCl 120 mM in the absence of COX inhibitors and in presence of indomethacin or mefenamic acid were respectively: $+144\pm25$, $+34\pm4$, and $+53\pm7.3$ mmHg ($n=6$).

Table 1 Difference in basal arterial (APP) and venous (VPP) perfusion pressure of mesenteries from WKY and SHRs, following perfusion of NOS and COX inhibitors

	APP		*VPP*	
	WKY	*SHR*	*WKY*	*SHR*
L-NAME	0.4 ± 0.7	0.4 ± 0.8	0.4 ± 0.2	0.7 ± 0.3
L-NOARG	-0.35 ± 0.1	-0.8 ± 0.2	0.2 ± 0.2	-0.1 ± 0.1
Indomethacin	-0.5 ± 0.2	-1.2 ± 0.4	0 ± 0.1	-0.1 ± 0.1
Mefenamic acid	-0.9 ± 0.7	-1.8 ± 0.7	-0.6 ± 0.3	-0.4 ± 0.4

Data are expressed in mmHg, and represent the mean ± s.e. mean ($n=6$).

In the mesenteric venous beds isolated from WKY, indomethacin had little effect on the increase in pressure induced by KCl (20 to 120 mM), but mefenamic acid diminished this response. The pressure values observed in control conditions and in the presence of indomethacin and mefenamic acid were, respectively, $+7.6\pm0.8$, $+5.8\pm0.5$ and $+2.5\pm0.2$ mmHg ($n=6$).

Acetylcholine-evoked relaxation of perfused arterial and venous beds derived from WKY and SHR mesenteric vasculature

Acetylcholine (1 nM to 10 μM) evoked concentration-dependent decreases in perfusion pressure of 100 mM KCl-contracted arterial and venous mesenteric beds taken from both groups of rats (Figure 4). In both groups of rats, the maximal relaxation evoked by acetylcholine (10 μM) was similar in the arterial compared to the venous beds, without a complete reduction in the induced tone (−80% maximum).

In the arterial mesenteric beds, the effects of the lower concentrations of ACh (1 and 10 nM) were significantly higher in preparations from SHR rats than from WKYs, while the effects of the higher concentrations of ACh (1 and 10 μM) were significantly smaller in SHR than WKY mesenteries.

In the venous mesenteric beds, the ACh-evoked relaxations were not significantly different between the two groups of rats, except at 10 nM, when expressed as % reduction in KCl contraction. When expressed as the difference in absolute pressure there were significant differences seen between the WKY and SHR at the higher concentrations of 1 and 10 μM ($P<0.001$).

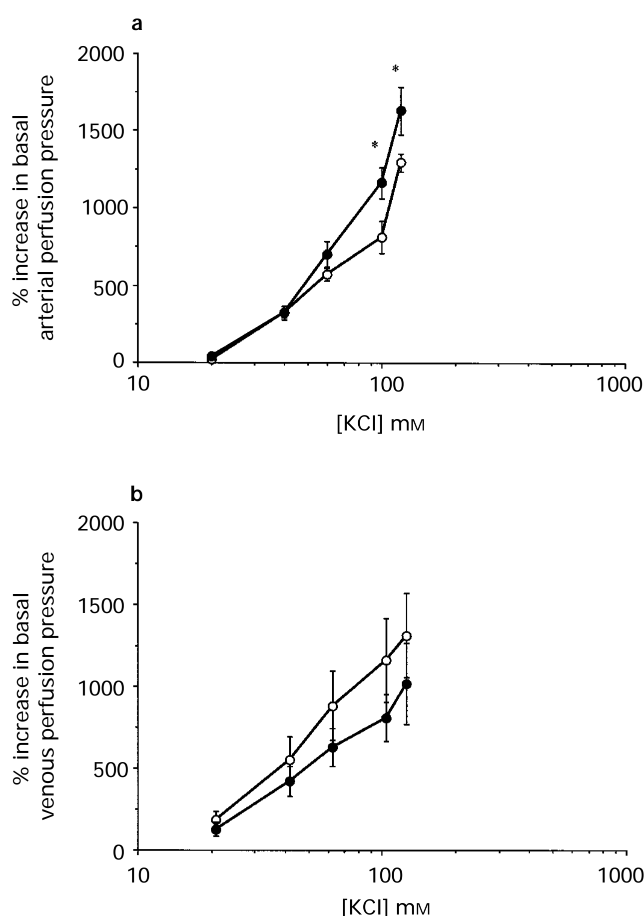

Figure 1 KCl (20 to 120 mM) concentration-response curves in perfused (a) arterial and (b) venous mesenteric beds isolated from (○) WKY and (●) SHR. Points show means and vertical lines indicate s.e.mean. *$P<0.05$ between the groups.

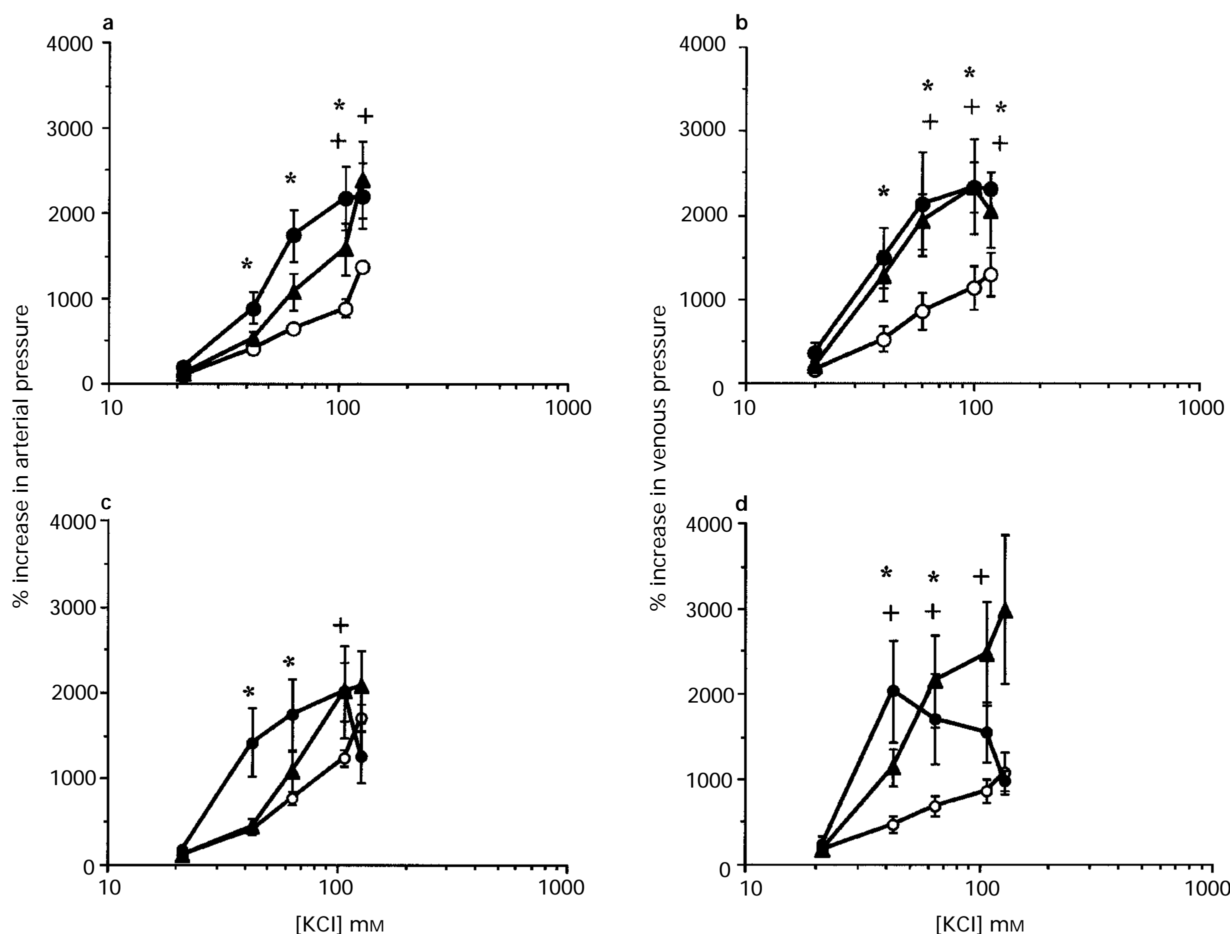

Figure 2 KCl (20 to 120 mM) concentration-response curves in perfused mesenteric (a and c) arterial and (b and d) venous beds, from (a and b) WKY and (c and d) SHR rats, in the (○) absence and presence of (●) 100 μM L-NAME or (▲) 100 μM L-NOARG. Points show means and vertical lines indicate s.e.mean. *$P<0.05$; $^{+}P<0.05$ significant changes from control.

Effect of L-NAME on acetylcholine-evoked vasodilatations of the perfused arterial and venous mesenteric vasculature beds

The effects of ACh were compared in vasculatures contracted with 100 mM KCl or with 40 mM KCl in combination with 100 μM L-NAME. Both high K^+ solutions induced the same contractions. For example in arterial preparations, the increases in tone due to 100 mM K^+ and 40 mM K^+ in combination with L-NAME were respectively $+24.5\pm5$ and $+33\pm6$ mmHg in WKY rats ($n=6$, NS). L-NAME completely inhibited ACh-induced relaxations in arterial and venous vasculatures isolated from WKY or SHR (results not presented).

Effect of mefenamic acid on acetylcholine-evoked relaxations of perfused arterial and venous mesenteric vascular beds

In arterial preparations, infusion of 5 μM mefenamic acid diminished the 100 mM K^+ induced tone, from 34.2 ± 4.4 to 28 ± 2 mmHg ($n=5$) in WKY rats but was without effect on the tone in preparations from SHRs ($n=6$).

Mefenamic acid was without effect on ACh-induced relaxations in either the arterial or the venous vasculatures isolated from WKY and SHR (results not presented).

Discussion

In the present study, with isolated mesenteric arterial and venous beds perfused with high K^+-Krebs solution, the relative importance of the NO-dependent and COX pathway in response to a non-receptor activator and in mediating ACh-relaxations from WKY and SHR was compared. Excess extracellular K^+ affects vascular smooth muscle membrane potential, allowing calcium entry into the cell. So, this mechanism is independent of the activation of a receptor, thus of a specific arterial and venous distribution of it, and facilates the comparison between arterial and venous beds. Moreover, an excess of extracellular K^+ decreases the influence of EDHF, which allowed us to study only the NO pathway.

We showed that there is no difference in the basal perfusion pressures of arterial and venous vessels within WKY and SHR mesenteric vasculature, despite the differences in mean arterial pressures of the two groups observed *in vivo*. Hence, vasculatures isolated from hypertensive rats responded to the same extent as vasculatures isolated from normotensive rats, when these vessels were perfused in the same conditions of flow. These results cannot be related to the higher mean arterial blood pressure observed *in vivo* and suggest that the responses of individual vascular beds may not be representative of the total peripheral resistance. Nevertheless, when the tone was increased by an excess of extracellular K^+, we observed a higher response in arterial beds isolated from SHR, but not in the venous ones. Hypertrophy of the media of large and small arteries of SHR has been established (Mulvany, 1990). Such structural changes may lead to a greater rise in arterial resistance, and thus to an increased response to KCl in the perfused arterial mesenteric bed. However, these results suggest that the venous mesenteric vasculatures isolated from SHR, do not seem to be modified.

Infusion of NOS inhibitors did not affect the basal arterial and venous perfusion pressures of either group, but induced an enhancement of KCl vasoconstriction, suggesting that NO was

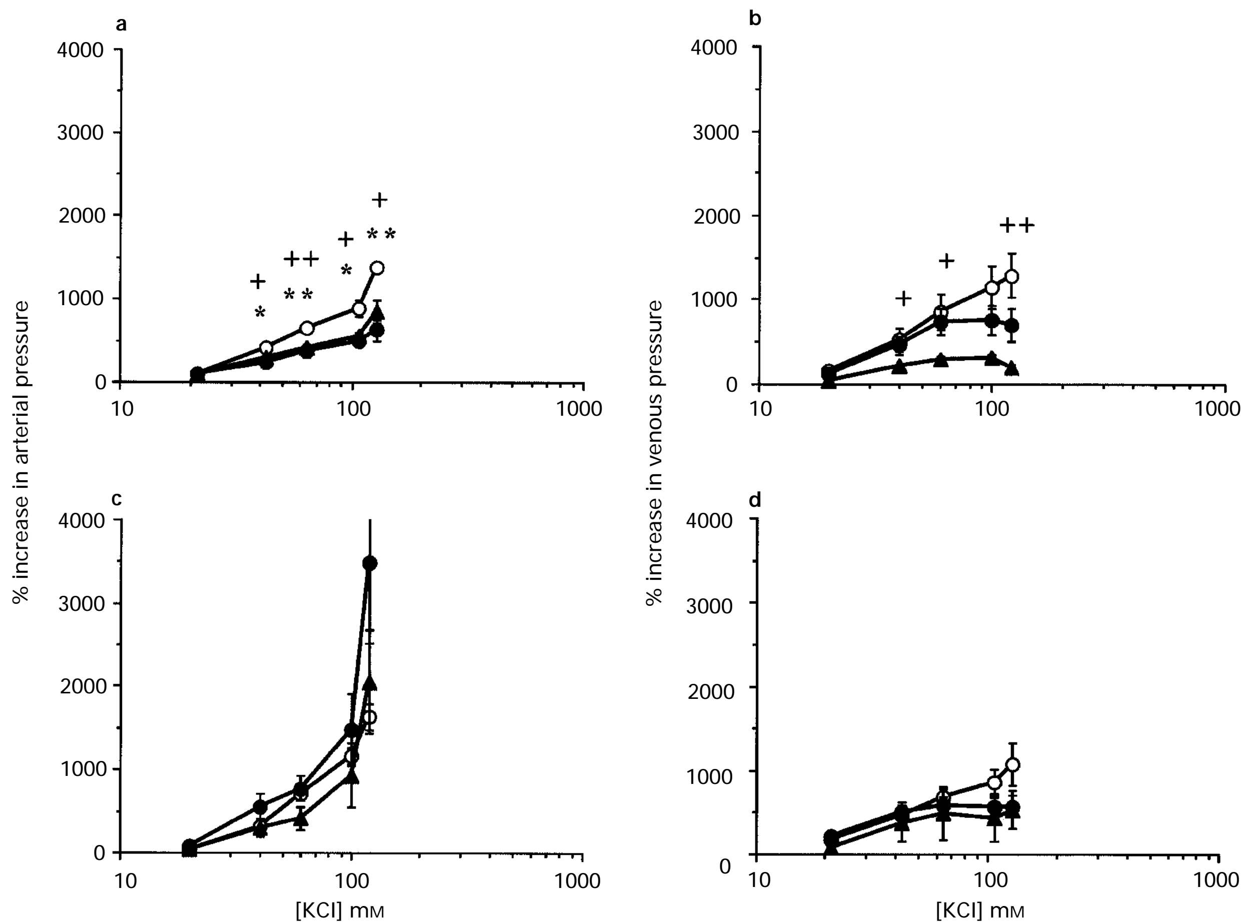

Figure 3 KCl (20 to 120 mM) concentration-response curves in perfused mesenteric (a and c) arterial and (b and d) venous beds from (a and b) WKY and (c and d) SHR rats, in the (○) absence and presence of (●) 5 μM indomethacin, or (▲) 5 μM mefenamic acid. Points show means and vertical lines indicate s.e.mean. $*P<0.05$; $**P<0.01$; $^{+}P<0.05$; $^{++}P<0.01$ significant changes from control.

not spontaneously released under our basal perfusion pressure conditions, and that liberation of NO requires an active tone mediated by an increase in intracellular Ca^{2+} induced by vasoconstrictors. This is in agreement with other studies performed in the mesenteric arterial beds of rats (Adeagbo *et al.*, 1994), which showed that no pressor response was observed during an infusion of L-NAME. L-NAME was still without effect despite the assumed increase in shear stress, resulting from an increase in the perfusion rate, supporting the conclusion that in the mesenteric bed NO is less important than EDHF.

There were no apparent differences in the potentiation of pressor effects caused by NOS inhibitors between the two groups of rats, indicating that there is no apparent dysfunction of NO synthase activity in the perfused mesenteric vessels of the SHR. Thus, the higher effect observed with KCl perfusion could be due to an increase in the release of a contracting factor, as suggested by Lüscher (1990), or structural changes making SHR vessels more responsive to contractile agonists.

COX inhibitors also had no effect on the basal arterial perfusion pressure in either group, but mefenamic acid induced a significant decrease in the venous perfusion pressure of mesenteries isolated from WKY rats, but not those from SHRs. Moreover, COX inhibitors induced a significant decrease in the arterial and venous response to KCl in WKY mesenteries, but not in those from SHRs. These results suggest that a cyclo-oxygenase-dependent contracting factor is spontaneously released under basal conditions in the perfused venous mesentery isolated from WKY but not from SHR. Similarly, in perfused arterial and venous mesenteric vasculatures isolated from WKY rats, but not SHRs, a cyclo-oxygenase-derived contracting factor, which enhances the arterial and venous contractions to KCl may also be formed.

Perfusion of K^{+} in the arterial and venous vasculatures isolated from WKY rats, most probably induced, therefore the liberation of NO together with a cyclo-oxygenase contracting factor. In arterial and venous mesenteric vasculatures isolated from SHR rats, the perfusion of K^{+} induced only the liberation of NO.

ACh evoked similar concentration-dependent relaxations in mesenteric arterial and venous vasculatures isolated from WKY rats, suggesting there is no apparent difference in NO-dependent responses between the two circulations. Removal of the endothelial cells with CHAPS abolished the relaxation, indicating that these responses were indeed endothelium-dependent. In a previous study on the perfused arterial mesenteric bed, L-NOARG was found to abolish ACh-evoked relaxations (Moore *et al.*, 1990). In the arterial and venous vasculatures precontracted with K^{+}, ACh-induced relaxations were totally inhibited by L-NAME, suggesting that these responses were mediated solely by endothelium-derived NO and indicating the relative importance of the liberation of NO in the venous compared to the arterial vasculature. These results do not accord with those from previous studies made *in vivo* by De Mey and Vanhoutte (1982) who observed, in veins, only moderate endothelium-dependent relaxations, but could perhaps be explained in part by the retrograde perfusion or by regional differences in apparent sensitivity. Together our data indicate that the relative contribution made by both NO-dependent and -independent mechanisms varies between vessels. Moreover, the COX pathway did not seem to be im-

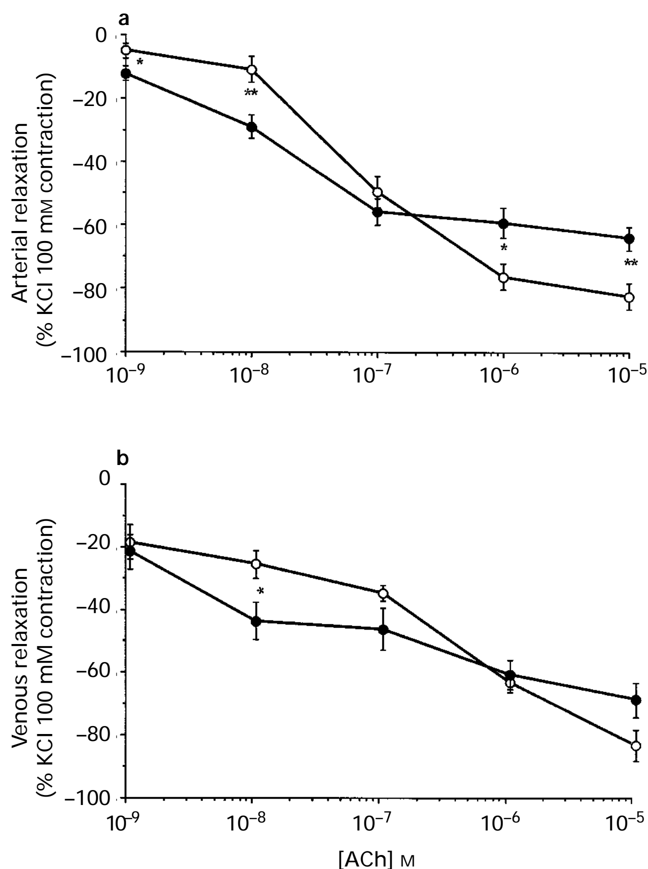

Figure 4 Acetylcholine (1 nM to 10 μM) concentration-response curves in perfused (a) arterial and (b) venous mesenteric beds isolated from (○) WKY and (●) SHR. Points show means and vertical lines indicate s.e.mean. $*P<0.05$; $**P<0.01$ between the

plicated, since the cyclo-oxygenase inhibitor, mefenamic acid, did not affect relaxations in either the arterial or venous mesenteric vasculatures. These results are coherent with those of Garland & McPherson (1992) and Takase *et al.* (1994).

In mesenteries isolated from SHRs, endothelium-dependent relaxations to high concentrations of ACh were altered in arterial beds compared to those in WKY mesenteric vasculatures, while the effects of low concentrations were not affected. In the venous bed of SHRs, we did not observe any difference in the action of ACh compared to WKYs. However, in KCl-precontracted arterial beds, conditions eliminating the action of EDHF, the relaxations to ACh were less impaired than in NA-precontracted vessels (Tesfamariam & Halpern, 1988), suggesting that endothelium-dependent relaxations in SHR are not impaired under all experimental conditions. It has been suggested that the impaired agonist-induced relaxations in hypertensive rats could be attributed to attenuated endothelium-dependent hyperpolarization (Kähönen *et al.*, 1995), which could explain the smaller impairment observed in the condition of depolarized vessels. Moreover, a cyclo-oxygenase-dependent mechanism is not implicated in the ACh-induced relaxations in mesenteric arterial or venous beds isolated from WKY or SHRs, as COX inhibitors had no effect on these relaxations. These results are in contradiction with those from other studies performed in isolated aortic rings from SHR, which have shown an impairment of endothelium-dependent relaxation, associated with an increased release of endothelium-derived thromboxane A_2 (TXA_2), a factor underlying the endothelial cell dysfunction (Kato *et al.*, 1990). Thus our data suggest that the type of vessel studied (resistance and not capacitive vessel) and the presence of perfusion which induces a shear stress, could modify the role of endothelium-derived cyclo-oxygenase.

In conclusion, the liberation of NO in the perfused mesenteric vasculatures required an active tone or circulating vasoconstrictors. The higher effect observed with KCl perfusion in the perfused mesenteric vessels of the SHR, is not due to a dysfunction of NO synthase activity, but probably as a result of an increase in the release of a contracting factor or structural changes making SHR vessels more responsive to contractile agonists. Perfusion of K^+ in the arterial and venous vasculatures isolated from WKY rats, most probably induced the liberation of NO together with a cyclo-oxygenase contracting factor, but induced only the liberation of NO in arterial and venous mesenteric vasculatures isolated from SHR rats. The relative contribution made by both NO-dependent and -independent mechanisms, which are not prostaglandin-mediated, varies between vessels. In the perfused arterial and venous mesenteric vasculatures isolated from SHR rats, the role of NO appeared to be preserved, while the COX pathway was not involved in the KCl-contraction, or in the ACh-induced relaxation.

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