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Flow dependence of nitric oxide-mediated pressure change in rat mesenteric beds with different tonus

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Abstract

To investigate the flow-dependent contribution of basally released nitric oxide (NO) to vascular perfusion pressure, we compared the effects of a NO synthesis inhibitor on the pressure changes in some models of rate mesenteric vascular beds. Spontaneously hypertensive (SHR) and normotensive Wistar Kyoto (WKY) rats (13–14-weeks-old) were used. The perfusion pressure at each flow rate was slightly higher in the SHR bed than in the WKY bed when no contractile compounds were applied. N^G-Monomethyl-L-arginine (L-NMMA) significantly increased the pressure in WKY at flow rates more than 5 ml/min (8.9 mm Hg at 7 ml/min). L-NMMA increased the pressure in SHR at flow rates of 2–7 ml/min (40.2 mm Hg at 7 ml/min). L-NMMA markedly increased the pressure at each flow rate in both beds which methoxamine (30 μM) had constricted. The effects of L-NMMA were concentration-dependent, and were blocked by L-arginine. Therefore, basal NO release appears to contribute to the vasolidating tone although flow dependence of the effect is different in the absence or presence of an exogeneous tone in both hypertensive and normotensive rats.

Keywords: Nitric oxide (NO); Mesenteric vascular bed; Flow; Spontaneously hypertensive rat (SHR)

1. Introduction

The biological activity of endothelium-derived relaxing factor (EDRF), which is known to be nitric oxide (NO) or a NO-containing moiety, has been confirmed (Lowenstein et al., 1994). L-Arginine analogs, such as N^G-monomethyl-L-arginine (L-NMMA), inhibit endothelium-dependent relaxation of isolated arteries, and this effect can be reversed by coincubation with L-arginine (Rees et al., 1990). Systemic administration of such analogs increases the arterial pressure and regional vascular resistance in several animal preparations, suggesting that a basal endothelial release of NO maintains vasodilating tone in blood vessels (Rees et al., 1989; Aisaka et al., 1989; Gardiner et al., 1990; Yamazaki et al., 1991). A mechanical stimulus, shear stress, has been shown to play some role in the basal NO release (Rubanyi et al., 1986), and to have a pathophysiological influence on blood pressure modulation in hypertension through this mechanism (Randall et al., 1991). Thus, flow rate, a determinant of shear stress, may cause the NO-mediated perfusion pressure change in the hypertensive as well as the normotensive state.

To date, endothelial dysfunction has been shown to occur in isolated large arteries of hypertensive rats (Konishi and Su, 1983; Winquist et al., 1984; Van de Voorde and Leusen, 1986). However, studies of the flow-mediated dilator mechanism have yielded contradictory results (Laurent et al., 1990; Randall et al., 1991; Panza et al., 1993; Koller and Huang, 1994; Qiu et al., 1994). Therefore, the question has arisen as to what extent flow rate affects the NO-mediated dilator response in hypertensive and normotensive states.

The purpose of the present study was to investigate the NO-mediated pressure changes in response to flow rates under several conditions, using isolated vascular beds from hypertensive and normotensive animals in the absence or presence of exogeneous vascular tone. To this end, we compared the effects of a NO synthesis inhibitor on the pressure changes in the mesenteric vascular beds of spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY), unconstricted by any contractile

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compound. In addition, we investigated the effects of the same inhibitor on the pressure changes in these vascular beds under conditions of higher tone induced by perfusion with methoxamine.

2. Materials and methods

2.1. Preparation

Rat mesenteric vascular beds were prepared using the method of McGregor (1965) modified as described by Moore et al. (1990). The present experiments were performed in accordance with the guiding principles for the care and use of laboratory animals approved by the Japanese Pharmacological Society. Male 13–14-week-old SHR and WKY (Charles River Japan) were used. At this age, the systolic arterial pressure, measured by a tail-cuff method, of conscious SHR was significantly higher (206 ± 6 mm Hg) than that of conscious WKY (154 ± 5 mm Hg; $P < 0.001$), whereas the heart rates were not significantly different (423 ± 18 and 431 ± 18 beats/min, respectively). The rats were killed by cervical dislocation. The mesenteric vascular beds were isolated by cutting close to the intestinal border of the mesentery from the rectum to the duodenum. The ileo-colic and duodenal branches of the superior mesenteric artery were tied, the mesenteric artery was separated from the surrounding tissues, cannulated with a polystyrene tube, and perfused with Krebs-Henseleit solution aerated with 95%(v/v) O_2-5%(v/v) CO_2 at a constant temperature of 32°C at a flow rate of 2 ml/min using a peristaltic pump (SJ-1211, Atto, Japan) for 1 h. The composition of the solution (mM) was: 118 NaCl, 24.6 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 1.2 $MgSO_4$, and 5.6 D-glucose, to which indomethacin (10 μM) was added to prevent cyclooxygenase product synthesis. The perfusion pressure was monitored continuously by means of a pressure transducer (TNF-R, Spectramed) connected to the side-arm of the cannula at the same hydrostatic level as the vascular bed. A compliance chamber was connected to the side-arm of the perfusion tube to reduce flow fluctuation. The electrical signal was amplified (AP-620, Nihon Kohden, Japan) and recorded (FBR-252A, Toa Dempa, Japan).

2.2. Protocols

Before the tissue was connected to the cannula, the pressure drop across the perfusion tube at each flow rate was measured, and the actual pressure at each flow rate was determined by subtracting the corresponding pressure drop across the perfusion tube from the recorded pressure. After the stabilization period, the flow rate was increased to 3 or 5 ml/min. All compounds were introduced into the vascular bed by perfusion. The perfusion was continued until its effect became stable. The concentration-response curves for the vasolidator response to acetylcholine and sodium nitroprusside were analysed by fitting the logistic equation: $R = (R_{max} \times A^{nH})/(EC_{50}^{nH} + A^{nH})$, where R is the amplitude of relaxation, R_{max} the maximal relaxation, A the concentration of acetylcholine or sodium nitroprusside, EC_{50} the dose of acetylcholine or sodium nitroprusside giving half-maximal relaxation, and nH is the slope function. For the pressure/flow experiment, the flow rate was raised stepwise from 2 to 7 ml/min in increments of 1 ml/min.

2.3. Materials

The drugs used were: L-NMMA acetate (donated by Tanabe Seiyaku Co., Japan); L-arginine HCl, D-arginine HCl and sodium nitroprusside (Nacalai Tesque, Japan); methoxamine HCl and indomethacin (Sigma, MO) and acetylcholine Cl (Ovisot; Daiichi Pharmaceutical Co., Japan). A stock solution of indomethacin (10 mM) was produced by dissolving it in ethanol, and diluted in the Krebs-Henseleit solution. The other compounds were dissolved in the Krebs-Henseleit solution.

2.4. Statistics

The data are expressed as means ± S.E.M.. Student's or Welch's t-test was used for statistical comparisons. Differences with $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Vasodilatator responses to acetylcholine and sodium nitroprusside

The baseline perfusion pressures in the mesenteric vascular beds of SHR and WKY at a flow rate of 5 ml/min were 28.6 ± 1.1 and 22.3 ± 0.7 mm Hg (both $n = 4$), respectively. In order to raise the tone of each to a similar level, 10–20 and 30–40 μM methoxamine was infused into the vascular beds of SHR and WKY, respectively. During the perfusion of methoxamine, the mean perfusion pressures were 65.2 ± 4.8 and 60.3 ± 2.4 mm Hg, respectively. Acetylcholine reduced the perfusion pressure in a concentration-related manner (Fig. 1A). Maximal vasodilation of both beds was evoked by 100 nM acetylcholine: the maximal values were 104 ± 1% and 105 ± 1% (both $n = 4$) of the methoxamine-induced tone in SHR and WKY, respectively. The effects of acetylcholine at any concentration in SHR were not significantly different from those in WKY. The EC_{50} values for acetylcholine in SHR (3.08 ± 0.35 nM) were similar to those in WKY (4.18 ± 0.49 nM).

When the beds were maximally dilated by acetylcholine, an NO synthesis inhibitor, L-NMMA (300 μM), was infused (Fig. 1B). In WKY, L-NMMA significantly

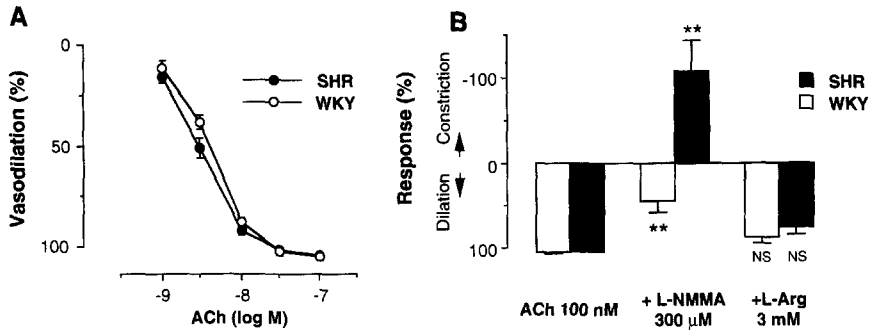

Fig. 1. Dilator effects of acetylcholine on methoxamine-pretreated mesenteric beds of SHR and WKY. The concentrations of methoxamine were 10–20 and 30–40 μM in SHR and WKY, respectively, which resulted in similar tone levels in these beds. Vasodilation was expressed as a percentage of the methoxamine-pretreated tone. (A) Comparison of acetylcholine-induced vasodilation in the two strains. No significant differences between the SHR and WKY groups were observed at any concentration. (B) Effects of subsequent infusion of L-NMMA and L-arginine (L-Arg). ** $P < 0.01$, compared with the acetylcholine (100 nM) group of the corresponding strain. After the infusion of L-NMMA in SHR, the tone overshot the level seen before the administration of acetylcholine.

($P < 0.05$) inhibited the effect of acetylcholine, reducing it to 44 ± 14% of the value before L-NMMA treatment. In SHR, this concentration of L-NMMA abolished the dilator response to acetylcholine, and furthermore caused marked vasoconstriction (107 ± 36%). When the L-NMMA-induced response had stabilized, L-arginine (3 mM) and L-NMMA were infused concomitantly. In both strains, L-arginine reversed the effect of L-NMMA and resulted in the same degree of vasodilation that was evoked by acetylcholine (100 nM) alone.

In another 4 preparations of each strain, sodium nitroprusside was infused into the methoxamine-pretreated beds. During infusion of 10–20 and 30–40 μM methoxamine into the beds of SHR and WKY, the mean perfusion pressures increased to a similar level, i.e. 62.7 ± 3.3 and 58.8 ± 3.5 mm Hg, respectively. Sodium nitroprusside caused concentration-related decreases in the perfusion pressures of both strains (Fig. 2), and the maximal vasodilation of both beds was evoked by 1 μM: the maximal values were 100 ± 3% ($n = 4$) of the methoxamine-induced tone in SHR and 95 ± 2% ($n = 4$) in WKY. The effect of sodium nitroprusside only at a concentration of 10 nM in SHR was greater than that in WKY. The EC_{50} values for sodium nitroprusside are 29.6 ± 2.8 nM in SHR and 102 ± 33 nM in WKY.

3.2. Vasoconstriction due to NO synthesis blockade

We examined the effect of L-NMMA (30, 100 and 300 μM) on the perfusion pressure in unconstricted vascular beds (no contractile compound was added) at a flow rate of 3 ml/min (Fig. 3). The basal pressure in SHR (15.1 ± 1.7 mm Hg, $n = 4$) was almost the same as that in WKY rats (12.4 ± 2.8 mm Hg, $n = 4$). In SHR, L-NMMA (30 and 100 μM) did not alter the perfusion pressure, although 300 μM L-NMMA increased it to 30.5 ± 6.0 mm Hg which was significantly greater ($P < 0.05$) than the basal pressure. This L-NMMA-induced increase was inhibited

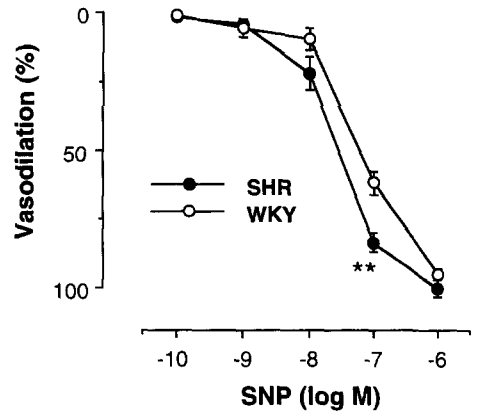

Fig. 2. Dilator effects of sodium nitroprusside on methoxamine-pretreated mesenteric beds of SHR and WKY, in which the concentrations of methoxamine were 10–20 and 30–40 μM, respectively, to produce similar levels of tone. Vasodilation was expressed as a percentage of the methoxamine-pretreated tone. ** $P < 0.01$, compared with the value at the corresponding concentration for the WKY group.

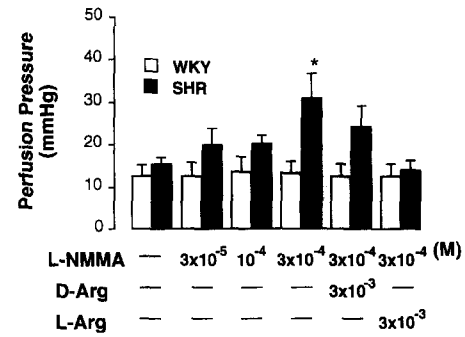

Fig. 3. L-NMMA-induced increases in the perfusion pressures of unconstricted mesenteric beds of SHR and WKY. Each compound, at the concentration indicated below the plots, was infused additively in the order shown from left to right. D-Arg, D-arginine; L-Arg, L-arginine. * $P < 0.05$, compared with the value at the corresponding concentration for the WKY group.

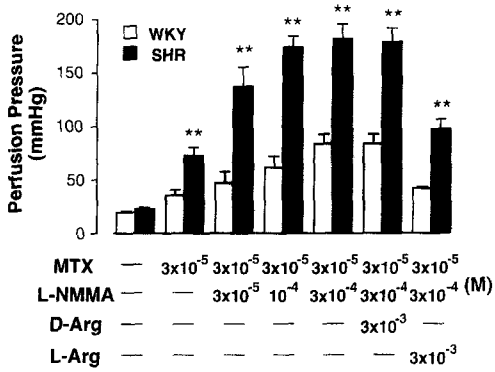

Fig. 4. L-NMMA-induced increases in the perfusion pressures of methoxamine-pretreated mesenteric beds of SHR and WKY. Each compound, at the concentration indicated below the plots, was infused additively in the order shown from left to right. D-Arg, D-arginine; L-Arg, L-arginine. * $P < 0.05$, ** $P < 0.01$, compared with the value at the corresponding concentration for the WKY group.

significantly ($P < 0.01$) by L-arginine (3 mM), but not by D-arginine (3 mM). None of these three concentrations of L-NMMA altered the perfusion pressure in WKY.

In methoxamine (30 μM)-preconstricted beds (Fig. 4), the basal pressure in SHR (72.2 ± 8.4, $n = 4$) was significantly ($P < 0.01$) higher than that in WKY (35.4 ± 5.6, $n = 4$), although little difference was observed between the two strains before the addition of methoxamine. In both strains, L-NMMA increased the perfusion pressure in a concentration-dependent manner. The perfusion pressure in each strain in the presence of 300 μM L-NMMA was significantly greater ($P < 0.01$) than that before addition of L-NMMA. The maximal perfusion pressure, which was evoked by 300 μM, was 2.1 times higher in SHR (181.3 ± 14.9 mm Hg, $n = 4$) than in WKY (84.4 ± 9.1 mm Hg, $n = 4$, $P < 0.01$). In the presence of 300 μM L-NMMA, 3 mM D-arginine had no significant effect, but the subsequent addition of 3 mM L-arginine reduced the perfusion pressure significantly ($P < 0.05$) to a level similar to that before addition of L-NMMA in both strains (SHR, 98.6 ± 8.6; WKY, 42.1 ± 1.1 mm Hg).

3.3. Flow dependence in unconstricted and methoxamine-pretreated beds

In the unconstricted vascular beds of both strains, the perfusion pressures increased when the flow rate was raised in a stepwise manner (Fig. 5A). The pressure/flow plots for both strains curved slightly towards the horizontal axis at the higher flow rates. The maximal perfusion pressure was 1.3 times higher in SHR (35.2 ± 1.7 mm Hg, $n = 5$) than WKY (27.3 ± 1.5 mm Hg, $n = 4$, $P < 0.05$), respectively. Infusion of L-NMMA (300 μM) increased pressure in both strains. It is noteworthy that the deviation from the control pressure/flow plot was greater for SHR than WKY. In WKY beds, the pressure/flow plots were shifted at flow rates higher than 5 ml/min. The maximal perfusion pressure was 2.0 times higher in SHR (75.4 ± 7.2 mm Hg) than WKY (36.2 ± 3.1 mm Hg, $P < 0.01$).

In the methoxamine (30 μM)-pretreated beds of SHR and WKY (Fig. 5B), the pressure increased linearly at flow rates of 2–7 ml/min, and was markedly higher at each flow rate than the corresponding unconstricted bed value. The maximal perfusion pressure in the methoxamine-pretreated beds was 2.2 times higher in SHR (121.2 ± 17.6 mm Hg, $n = 5$) than WKY (56.3 ± 9.3 mm Hg, $n = 4$, $P < 0.05$). Infusion of L-NMMA (300 μM) increased the perfusion pressure markedly in both strains. In the SHR

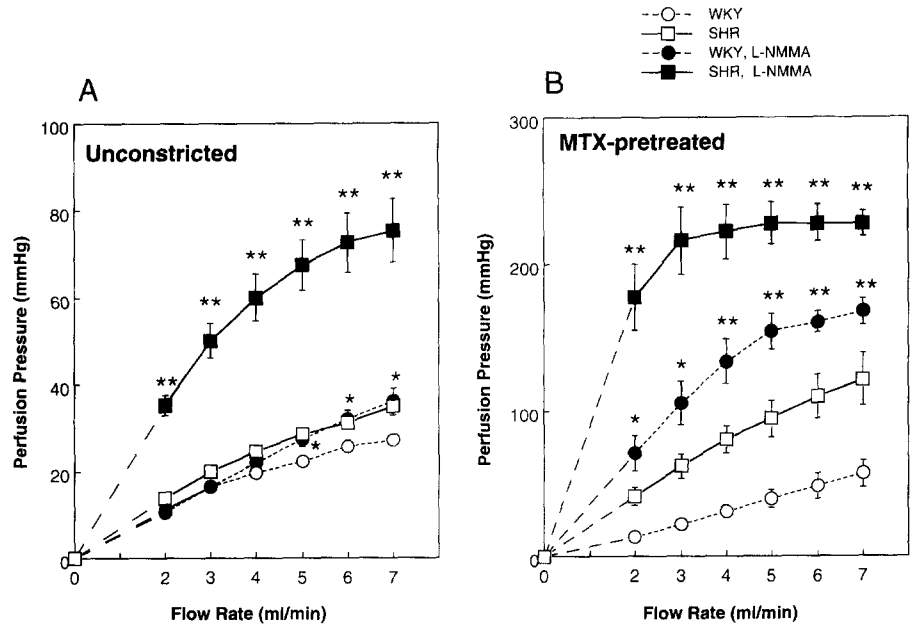

Fig. 5. Perfusion pressure-flow rate (pressure/flow) relationships for the unconstricted (A) and the methoxamine-pretreated (B) mesenteric beds of SHR and WKY. The flow rate was increased in a stepwise manner. The pressure drop across the perfused bed in the presence and absence of 300 μM L-NMMA is shown. * $P < 0.05$, ** $P < 0.01$, compared with the value in the absence of L-NMMA.

beds, the perfusion pressure was very high, even at flow rates below 3 ml/min, and it increased moderately to a constant level (approximately 228 mm Hg) as the flow rate increased. In the WKY beds, the perfusion pressure increased as the flow rate increased, but the pressure/flow plots were less linear than those for the unconstricted beds. The maximal perfusion pressure was 1.4 times higher in SHR than WKY ($P < 0.01$).

4. Discussion

In the present study, we focused on the flow-dependent contribution of basally released NO to the vascular perfusion pressure in the mesenteric vascular bed of two strains of rats in the presence or absence of an exogeneous tone. First, we examined the validity of the present preparation and a NO synthesis inhibitor. We investigated the responsiveness of the mesenteric vascular bed to an endothelium-dependent vasodilator and found that the acetylcholine-induced dilator response in SHR was similar to that in WKY. This result is consistent with the findings of studies on perfused vasculature (Randall et al., 1991; Koller and Huang, 1994). A cyclooxygenase inhibitor, indomethacin, was used throughout the present study so that the participation of prostanoids could be ruled out.

An inhibitor of NO synthesis, L-NMMA, at the concentration that partially inhibited vasodilation in WKY, abolished acetylcholine-induced dilation and furthermore, evoked vasoconstriction in SHR. This action of L-NMMA was specific to NO, as it was reversed by L-arginine. The concentration of L-NMMA (300 μM) used in this study is known to be high enough to block NO-mediated vasodilation in perfused preparations (Griffith and Edwards, 1990; Fukuda et al., 1992). Therefore, the remaining relaxation in the presense of L-NMMA may be due to the other endothelium-dependent substances. Adeagbo and Triggle (1993) reported that another inhibitor of NO synthesis, N^G-nitro-L-arginine methylester, only partially blocked vasodilation in response to acetylcholine in rat mesenteric beds, and that the remaining dilation was abolished in a high-K^+ solution. Together, these findings indicate that acetylcholine may release both NO and endothelium-derived hyperpolarizing factor (EDHF) from the vascular beds in WKY. The interesting phenomenon that L-NMMA, furthermore, evoked vasoconstriction in SHR suggests the existence of basal dilatation, which is usually masked by strong constriction. Therefore, there may be more basal release of NO or a greater sensitivity of smooth muscles to NO in SHR. Once it has been effectively blocked by a NO synthesis inhibitor, the vascular tone appears to overshoot the level from before addition of acetylcholine.

We tried to determine the NO-dependent responses in the absence of an exogeneous tone by using unconstricted vascular beds from two strains of rats. L-NMMA increased the perfusion pressure in SHR markedly in a concentration-dependent manner at a flow rate of 3 ml/min and this effect was abolished by L-arginine. However, L-NMMA evoked little change in WKY, even at the highest concentration used (Fig. 3). These results suggest that, in the absence of exogeneous tone, NO is probably released by the unconstricted vascular bed of SHR, but not of WKY. However, NO may have been released preferentially under higher-flow conditions (higher than 4 ml/ml) in WKY. Therefore, we investigated the flow-induced effect on perfusion pressure in the vascular beds.

In the absence of L-NMMA, the pressure/flow lines for SHR and WKY curved slightly towards the horizontal axis at higher flow rates. This result may reflect passive distension of the blood vessels. When L-NMMA was applied to the WKY bed, the pressure/flow line was shifted at flow rates higher than 5 ml/min (Fig. 5). Therefore, the basal release of NO may be attributable to distension in WKY rats. In SHR, the flow-dependent contribution of NO to the pressure is markedly different from that in WKY, since the pressure/flow lines for SHR were shifted upward markedly, not only at high, but also at low flow rates in the presence of L-NMMA.

In the present study, we observed different shifts of pressure/flow lines and flow dependence in SHR and in WKY. These differences may be due to morphological alterations, such as medial hypertrophy. For example, medial thickening increases the wall-to-radius ratio, resulting in a decrease in luminal diameter. This phenomenon is considered to influence the constrictor response and to be essentially non-specific for overall vascular responsiveness (Folkow et al., 1970). Therefore, structural changes that occur in hypertension may also enhance the level of shear stress in vascular endothelium (Randall et al., 1991). In addition to the different basal release of NO in response to shear stress, other factors may also exist, one of which may be different sensitivities of the two strains to NO. While we compared the relaxation induced in both strains by sodium nitroprusside, a NO-liberating agent, we observed only a slight difference of sodium nitroprusside-induced effects between SHR and WKY under our experimental conditions.

Without L-NMMA, the perfusion pressure of methoxamine-constricted vascular beds increased as the flow rate rose and it was higher in SHR than WKY. These results agree with those reported previously (Koller and Huang, 1994). L-NMMA increased the perfusion pressure in a concentration-dependent manner in both strains and this effect was abolished by L-arginine. The results of the present and previous (Randall and Griffith, 1991) studies using an NO synthesis inhibitor suggest a contribution of released NO to vascular conductance in both beds with exogeneous tone. The L-NMMA-induced effects in SHR and WKY differed considerably. This was probably due to the different tones in the two strains under the present experimental conditions and/or a different intrinsic tone. This explanation was also discussed by Vargas et al.

(1990) whose in vivo study showed that the response to L-NMMA increased as the vascular tone increased.

The flow-dependent contribution of NO to the perfusion pressure was different between methoxamine-constricted and unconstricted vascular beds. When L-NMMA was applied to the unconstricted WKY bed, the pressure/flow line was shifted at flow rates higher than 5 ml/min but not less than 5 ml/min. On the other hand, in the methoxamine-pretreated beds, the pressure/flow plots were shifted at each flow rate in response to L-NMMA although there appears to have been a 'ceiling' effect above 200 mm Hg in SHR. In WKY, the perfusion pressure was 3–4 times higher in the presence than in the absence of L-NMMA at each flow rate. Therefore, we could not find any apparent flow-dependent contribution of NO to the perfusion pressure in methoxamine-constricted vascular beds. As the vascular tone was so high, the shear stress may have been large enough to cause the maximal dilator effect at the lowest flow rate. At the lower vascular tone, the contribution of basally released NO is likely to be more susceptible to flow rate.

It would appear that an effect of the basal release of NO is not necessarily observed at all the stages of hypertension development. Morphological studies indicated that intimal damage of the aorta of SHR develops predominantly from the age of 10 weeks, whereas medial damage in the aorta and peripheral arteries occurs from the age of 5 weeks (Limas et al., 1980). If the intima of large parts of the resistance arteries is not impaired, then the remaining NO release can partially compensate for the augmented vascular contractile function and this may oppose the augmented myogenic responses in hypertensive animals (Griffith and Edwards, 1990; Pohl et al., 1991).

In conclusion, the nitric oxide synthesis inhibitor constricts mesenteric vasculature with or without exogeneous tone in SHR and WKY. Flow rate may play some role in the effect of the inhibitor on the vascular tone, although flow dependence is different in SHR and in WKY. Thus, basal NO release appears to contribute to the vasodilating tone of the mesenteric beds in SHR and WKY, with a varying dependence on flow rates.

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