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Alterations of cyclo-oxygenase products and NO in responses to angiotensin II of resistance arteries from the spontaneously hypertensive rat

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1 The involvement of cyclo-oxygenase (COX) products and nitric oxide (NO) in contractile responses of resistance arteries to angiotensin II (AII) were investigated in small mesenteric arteries from spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats.

2 In endothelium intact vessels, AII induced concentration-dependent responses without any significant difference between the two strains. However, removal of functional endothelium resulted in enhanced sensitivity to AII, the pD_2 value increasing from 8.4 ± 0.2 to 8.9 ± 0.2 ($P<0.05$) in WKY and from 8.2 ± 0.1 to 8.6 ± 0.1 ($P<0.05$) in SHR (not significantly different between strains, $n=9-12$). In addition, endothelium removal enhanced maximal contractions elicited by AII in SHR (1.4 ± 0.1 to 2.1 ± 0.2 mN mm^{-1}, $n=5$; $P<0.01$) but not in WKY (1.0 ± 0.1 to 1.2 ± 0.1 mN mm^{-1}, $n=5$) vessels.

3 In the absence of functional endothelium, the COX inhibitor indomethacin (10^{-5} M) reduced contractile responses elicited by AII in SHR arteries, resulting in $33 \pm 5\%$ ($n=5$) decrease in maximal contraction. However, it produced minimal if any, effect on responses of WKY vessels. In both strains, the TP receptor antagonist GR32191 B (3×10^{-6} M) did not modify contractions elicited by AII in these conditions.

4 In the presence of functional endothelium, indomethacin (10^{-5} M) almost abolished the responses to AII in both strains. GR32191 B (3×10^{-6} M) reduced the sensitivity of WKY arteries to AII ($pD_2 = 8.1 \pm 0.1$, $P<0.01$) without any effect on maximal contraction. In SHR arteries, it markedly reduced maximal contraction ($47 \pm 3.5\%$).

5 In both strains, the NO synthase inhibitor N^G-nitro-L-arginine methy lester (L-NAME; 10^{-4} M) had no effect in the absence of functional endothelium but it markedly reduced the inhibitory influence of endothelium on contractile responses to AII. Furthermore, in arteries with endothelium, it reduced the effect of both indomethacin and GR32191 B to the same level as observed in vessels without functional endothelium.

6 The results suggest that enhanced contraction caused by COX products was counteracted by enhanced relaxation caused by endothelium-derived NO in resistance mesenteric arteries of the SHR exposed to AII, compared to WKY arteries. The COX products involved in alterations of SHR responses comprised an endothelium-derived prostaglandin activating TP receptors and another non-endothelial unidentified vasoconstrictor compound which did not activate these receptors.

Keywords: Endothelium; thromboxane A_2; prostaglandin H_2; TP receptor; AT_1 receptor; smooth muscle; Wistar Kyoto rat

Introduction

Angiotensin II (AII), the bioactive product of the renin-angiotensin system, has been shown to be involved in the onset of hypertension in the spontaneously hypertensive rat (SHR) since treatments with renin and angiotensin converting enzyme (ACE) inhibitors, and with nonpeptidic AII antagonists, can prevent the development of hypertension in this strain (Wong *et al.*, 1990; Wood *et al.*, 1990, reviewed by Timmermans *et al.*, 1993).

It has also been shown that responses produced by AII are increased in blood vessels as well as in cultured, smooth muscle cells from SHR (Bendhack *et al.*, 1992; Bodin *et al.*, 1993; Guidi & Hollenberg, 1987; Sugiyama *et al.*, 1990). This enhanced responsiveness has been variously attributed to increased AII receptor density (Bunkenburg *et al.*, 1992), enhanced phospholipase C activation (Resink *et al.*, 1989) and calcium storage (Levitsky *et al.*, 1993) in SHR vascular smooth muscle. In addition to its action on vascular smooth muscle, AII has been shown to stimulate endothelial production of vasoconstrictor factors such as cyclo-oxygenase (COX) products and endothelin in resistance arteries of SHR (Dohi *et al.*, 1992; Lang *et al.*, 1995). The production of contractile COX products by the endothelium was also observed in mesenteric arteries of Wistar rats contracted with noradrenaline or potassium (Wu *et al.*, 1994). Furthermore, endothelium-dependent relaxation is decreased (Dohi *et al.*, 1990; Watt & Thurston, 1989) and endothelium-dependent contraction is augmented (Diederich *et al.*, 1990; Takase *et al.*, 1994) in mesenteric arteries from SHR. Thus, altered responsiveness to AII in SHR is a complex mechanism which involves a combination of both vascular smooth muscle and endothelial dysfunction. However, most of the above studies were performed in conduit arteries. Few studies have been performed in resistance arteries which are involved in the regulation of blood pressure.

Therefore, the aim of the present study was to investigate the mechanisms involved in the alterations of contractile responses to AII in small mesenteric resistance arteries from SHR, and the participation of the endothelium in these phenomena.

Methods

Animals

Male SHR and WKY rats (12-14 weeks old) were bred in our institute from genitors provided by Ifa-Credo (Lyon, France).

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All rats were maintained in a colony room with fixed dark:light cycles of 12 h and constant humidity and temperature, and they were provided with rodent chow no. A04 from Usine d'Alimentation Rationnelle (Villemoisson, France) and tap water *ad libitum*. This investigation conforms with the authorisation (number 01918) for the use of laboratory animals given by the French government (Department of Agriculture). Systolic blood pressure was determined in conscious, restrained rats by the tail-cuff plethysmographic method (PE-300, Narco Biosystems Inc., Houston, USA). As expected, the mean systolic blood pressure of SHR was significantly higher than that of WKY being 182 ± 8 mmHg ($n = 12$) for SHR and 121 ± 7 mmHg ($n = 13$) for WKY, respectively ($P < 0.001$).

Arterial preparation and mounting

The animals were killed by cervical dislocation and exsanguinated. The viscera were exposed, a proximal segment of the small intestine was removed and pinned in a dissecting dish containing physiological salt solution (PSS) of the following composition (in mM): NaCl 119, KCl 4.7, KH_2PO_4 0.4, $NaHCO_3$ 14.9, $MgSO_4$ 1.17, $CaCl_2$ 2.5 and glucose 5.5. Branch II or III of superior mesenteric arteries were cleaned of fat and connective tissue, and a segment 1.6 to 2.0 mm in length was removed. In some experiments, the endothelial layer was removed immediately after dissection by intraluminal perfusion with 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1 propane sulfonate (CHAPS) in PSS for 25 s followed by repeated washing with PSS. The segment was then mounted on a previously described myograph (Mulvany & Halpern, 1977) with two tungsten wires (20 μm in diameter) inserted through the lumen of the vessel. Mechanical activity was recorded isometrically by a force transducer (Kistler-Morse, DSG BE4). After mounting, the vessel was placed in PSS, kept at 37°C and gassed continuously with 95% O_2 and 5% CO_2 (pH 7.4). After an equilibration period of 30 min, the vessel was stretched to a length that yields a circumference equivalent to 90% of that given by an internal pressure of 100 mmHg; this required a load of about 200 mg. After setting the vessel to its working length, two challenges with 10^{-5} M noradrenaline (NA) were performed to elicit reproducible contractile responses. The presence of functional endothelium was assessed by the ability of acetylcholine (ACh; 10^{-6} M) to induce more than 50% relaxation of vessels pre-contracted with NA (10^{-5} M). The absence of a relaxation response to ACh was taken as evidence that the vessel segments were functionally denuded of endothelium.

Relaxation and contraction experiments

Relaxation experiments were performed in arteries with functional endothelium. The effect of ACh was studied in vessels maximally pre-contracted with NA (10^{-5} M), then exposed to cumulative additions of ACh (from 10^{-9} to 10^{-6} M). It should be noted that higher concentrations of NA did not induce any further increase in contractile tension in arteries from both strains as previously shown by Andriantsitohaina *et al.* (1991).

In both endothelium intact and endothelium denuded arteries, concentration-response curves to AII were constructed by non-cumulative application of increasing concentrations of AII (10^{-9} to 10^{-7} M). Successive additions of different concentrations of AII were separated by 60 min washout periods. A partial depolarisation of the vessels with PSS containing 25 mM KCl was needed to obtain sustained and reproducible contractions with AII. Changing the bath from normal PSS containing 4.7 mM KCl to 25 mM KCl-PSS throughout the experiment did not produce any increase in contraction in vessels with and without endothelium. The influence of endothelial nitric oxide (NO) production, by use of the specific inhibitor of NO synthase N^G-nitro-L-arginine methyl ester (L-NAME), the involvement of COX, by use of the inhibitor indomethacin, and the implication of thromboxane (TP)-receptors, by use of the TP-receptor antagonist GR32191 B, were respectively investigated. All the inhibitors and antagonists were used at maximally active concentrations (i.e., 10^{-4} M for L-NAME, 10^{-5}M for indomethacin, 3×10^{-6} M for GR32191 B) and were added 30 min before AII. Preliminary experiments showed that higher concentrations of L-NAME, indomethacin and GR32191 B did not produce greater inhibitory effects in arteries from either strain.

Drugs

AII, CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1 propane sulphonate), L-NAME (N^G-nitro L-arginine methyl ester hydrochloride), acetylcholine chloride, indomethacin, quinacrine, cocaine and noradrenaline bitartrate were purchased from Sigma (St Quentin Fallavier, France). Losartan was provided by Dupont de Nemours (Wilmington, U.S.A.). GR32191 B ([1R-[1α (z), 2β, 3βm 5α]]-(+)-7-[5-[[(1,1'-biphenyl)-4-yl] methoxy] -3- hydroxy -2- (1-piperidinyl) cyclopentyl] -4- heptenoic acid, hydrochloride) was a generous gift from Glaxo Research and Development (Hertfordshire, U.K.).

Data analysis

Active wall tension is expressed in mN mm^{-1}. The sensitivity to AII is expressed as the pD_2 value, where pD_2 represents $-\log$ of the half maximally effective molar concentration (EC_{50}). All results are shown as mean $\pm$ s.e. mean, and n denotes the number of experiments. All curves were fitted by dose-response nonlinear regression equation using the Graphpad Prism program (San Diego, U.S.A.). Multivariate analysis of variance (MANOVA) was used for statistical analysis with the SYSTAT program. The statistical analysis of the maximal AII contractile effect and pD_2 values was determined by the Mann-Whitney test. P values < 0.05 were considered significant.

Results

Effect of endothelium

Concentration-effect curves for AII were constructed in arteries from both SHR and WKY rats, with and without functional endothelium. The mean pD_2 values corresponding to these experiments are shown in Table 1. The responses obtained with AII (10^{-7} M) were considered as maximal because, in preliminary experiments, increasing AII concentration above 10^{-7}M did not induce any further increase in contractile tension. On the contrary, it caused rapid fading of the response of arteries with endothelium from WKY and SHR, as previously found in Wistar rat small mesenteric arteries (Andriantsitohaina *et al.*, 1996).

Comparison of the results obtained in WKY (Figure 1a) and SHR (Figure 1b) vessels showed no significant strain difference in the responses of arteries with functional endothelium (MANOVA, entire curve). As shown in Figure 1, removal of the endothelium significantly enhanced contractile responses to AII in the two strains (MANOVA: $P < 0.05$ in WKY and $P < 0.01$ in SHR). This resulted in an increase in pD_2 values (Table 1). However, the maximal contractile tension induced by AII (10^{-7}M) was significantly enhanced by endothelium denudation in SHR only ($P < 0.01$). The contractile responses to AII of arteries without endothelium were significantly higher in SHR, compared to WKY (MANOVA: $P < 0.001$). Thus, removal of endothelium produced a larger increase in contractile responses elicited by AII in SHR than in WKY arteries.

In vessels from both strains, with or without endothelium, the response induced by the maximal concentration of AII (10^{-7}M) was abolished by the AT_1-receptor antagonist losartan 10^{-6}M (tension reached in the presence of losartan: 0.12 ± 0.07 and 0.10 ± 0.05 mN mm^{-1} in SHR vessels and 0.07 ± 0.05 and 0.09 ± 0.05 mN mm^{-1} in WKY vessels with and without functional endothelium, respectively, $n = 4$).

Table 1 pD2 values to angiotensin II (AII) in resistance arteries from WKY and SHR

	WKY		*SHR*	
	+ Endothelium	*− Endothelium*	*+ Endothelium*	*− Endothelium*
Control	8.4±0.2 (10)	8.9±0.2 (9)†	8.2±0.1 (12)	8.6±0.1 (10)†
L-NAME (10^{-4} M)	8.8±0.3 (5)	9.0±0.3 (4)	8.7±0.1 (5)**	8.8±0.1 (5)
Indomethacin (10^{-5} M)	ND	ND	ND	8.2±0.2 (5)
GR32191 B (3×10^{-6} M)	8.1±0.1 (5)	9.1±0.1 (5)††	ND	8.4±0.1 (5)
L-NAME (10^{-4} M) + indomethacin (10^{-5} M)	8.2±0.2 (5)	ND	8.1±0.2 (5)	ND
L-NAME (10^{-4} M) + GR32191 B (3×10^{-6} M)	8.7±0.2 (5)	ND	8.0±0.2 (5)‡#	ND

Values are mean±s.e.mean with number of observations shown in parentheses. **$P<0.01$ versus control values; †$P<0.05$ and ††$P<0.01$ versus +endothelium values; ‡$P<0.05$ and ‡‡$P<0.01$ versus WKY values and #$P<0.01$ versus L-NAME values, by Mann-Whitney test. ND, could not be determined (see text).

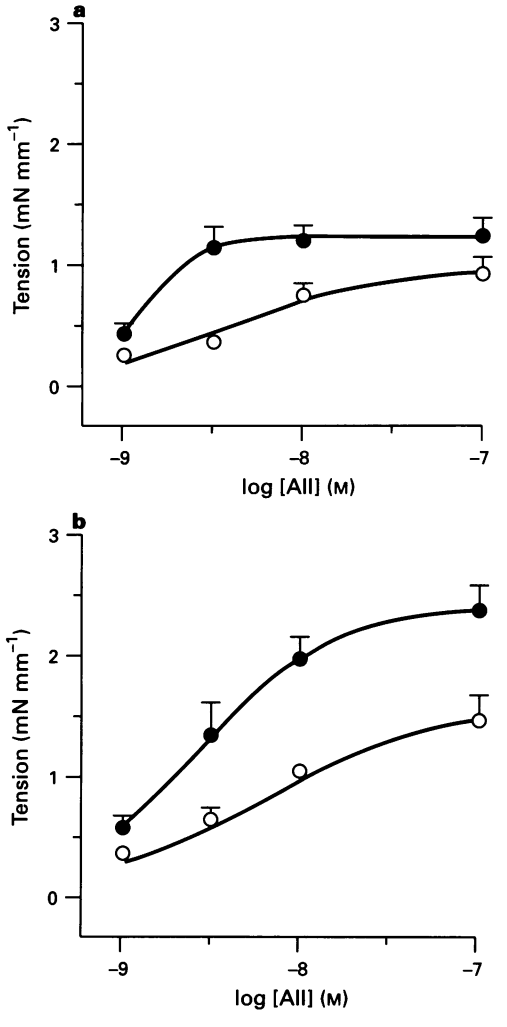

Figure 1 Concentration-response curves to angiotensin II (AII) of rat small mesenteric resistance arteries with (○) or without (●) functional endothelium taken from WKY (a) or SHR (b). The values are mean of 5 and 7 experiments for concentration-response curves to AII in WKY and SHR, respectively; vertical lines show s.e. mean.

The endothelium-dependent effects of ACh previously described in small mesenteric arteries of the SHR and the WKY rat (Tesfamariam & Halpern, 1988; Watt & Thurston, 1989) were also verified (Figure 2). As expected, ACh at concentrations up to 10^{-5} M produced concentration-dependent relaxation in WKY arteries. In contrast, the concentration-effect curve to ACh was biphasic in SHR arteries, comprising a relaxing component (for ACh concentrations $\leq 3\times10^{-7}$ M) and a further vasoconstrictor component at concentrations higher than 3×10^{-7} M ($n=5$, Figure 2).

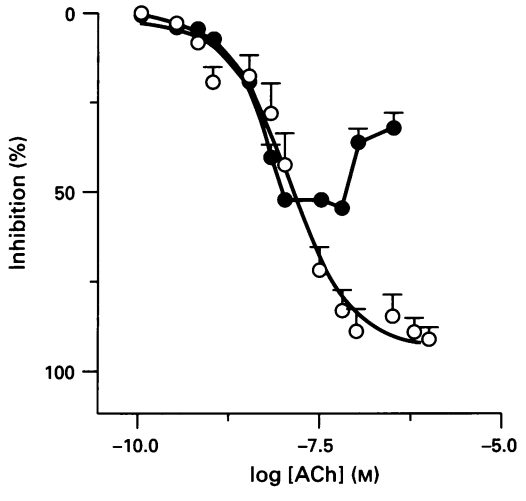

Figure 2 Concentration-response curves to acetylcholine (ACh) in rat mesenteric resistance arteries with functional endothelium pre-contracted with noradrenaline (NA, 10^{-5} M) taken from WKY (○) or SHR (●). Values are mean of 5 experiments; vertical lines show s.e. mean.

Effects of L-NAME, indomethacin and GR32191 B in endothelium-denuded arteries

As expected, L-NAME (10^{-4} M) had no effect on AII-induced contractions in endothelium-denuded arteries from either strain (not shown), resulting in unchanged pD_2 values (Table 1). Indomethacin (10^{-5} M) markedly attenuated the response of SHR vessels without endothelium (MANOVA: $P<0.05$; Figure 3b): its effect on the pD_2 value was not statistically significant (Table 1), but the maximal response was attenuated ($P<0.05$). By contrast, it did not produce any effect in endothelium-denuded arteries from WKY rats (Figure 3a). In the latter case, the concentration-effect curve was too flat to permit reasonable determination of pD_2 values. In the absence of functional endothelium, GR32191 B (3×10^{-6}M) did not modify contractile responses elicited by AII in either SHR or WKY vessels (Table 1).

Effects of L-NAME, indomethacin and GR32191 B in arteries with endothelium

L-NAME (10^{-4} M) enhanced contractile responses to AII of vessels with functional endothelium from either strain (MANOVA: $P<0.05$ in WKY and $P<0.01$ in SHR) and it decreased the corresponding pD_2 values (Table 1). Concentration-effect curves obtained in the presence of L-NAME in these arteries (Figure 4) were indistinguishable from those obtained in endothelium-denuded vessels (Figure 1), resulting in pD_2 values which were not different from those obtained in control arteries without endothelium (Table 1).

In vessels with endothelium from either strain, contractile responses to AII were almost completely abolished in the presence of indomethacin (10^{-5} M) alone (Figure 4a and b). Accordingly, a pD_2 value could not be calculated. In the presence of L-NAME, indomethacin also significantly attenuated the effect of AII (MANOVA: $P<0.05$ in WKY and $P<0.01$ in SHR), although the depression of the contractile responses was much less marked than in the absence of L-NAME (Figure 4a and b). In the presence of L-NAME (Figure 4), as in endothelium-denuded vessels (Figure 3), the effect of indomethacin was greater in SHR than in WKY arteries. Contractile responses elicited by AII in arteries with functional endothelium were not significantly modified by simultaneous exposure to L-NAME and indomethacin (MANOVA). This is illustrated in Figure 4b, where concentration-effect curves to AII are superimposed in the case of SHR vessels, in the absence and in the presence of both L-NAME and indomethacin. In the case of WKY vessels (Figure 4a) the maximal effect induced by AII (10^{-7}M) was slightly but significantly larger in vessels exposed to L-NAME plus indomethacin ($P<0.05$). In the presence of L-NAME alone, contractile responses to AII were greater in tissues from SHR than in those from WKY (MANOVA: $P<0.05$). Thus, the enhancing effect of L-NAME entirely neutralised (in SHR) or even partially reversed (in WKY) the inhibitory effect of indomethacin in arteries with functional endothelium.

As shown in Figure 5, GR32191 B significantly decreased the response to AII of arteries with functional endothelium from either WKY rats (MANOVA: $P<0.05$) or SHR (MANOVA: $P<0.001$). However, contractile tension was more depressed in SHR vessels (in which the maximal response was markedly blunted) than in WKY vessels (in which the maximal response was not decreased). The pD_2 value of AII was not calculated in the case of GR32191 B exposed SHR arteries (Table 1), because of the large impairment of contractile responses. In the presence of L-NAME, GR32191 B significantly attenuated the effect of AII only in vessels from SHR (MANOVA: $P<0.05$; Table 1). The effect of the simultaneous exposure of L-NAME and GR32191 B was similar to that observed in vessels exposed to indomethacin in the presence of L-NAME, as the contractile responses elicited by AII in these conditions were not significantly different from those obtained in control arteries with functional endothelium (MANOVA), from either strain (Table 1).

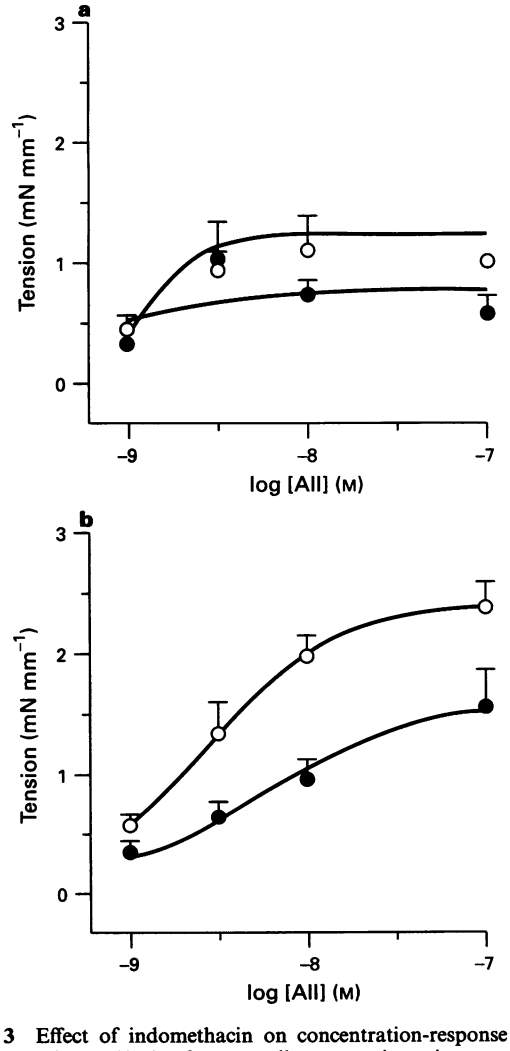

Figure 3 Effect of indomethacin on concentration-response curves to angiotensin II (AII) of rat small mesenteric resistance arteries without functional endothelium taken from WKY (a) or SHR (b). The values are mean of 6 or 5 experiments, in the absence (○) or presence of indomethacin (10^{-5}M; ●), respectively; vertical lines show s.e. mean.

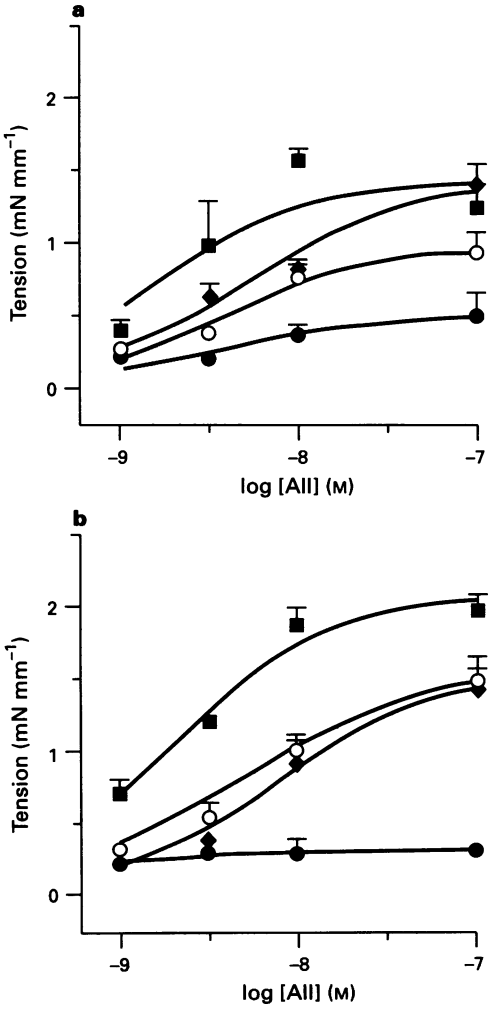

Figure 4 Effect of L-NAME, indomethacin and L-NAME plus indomethacin on concentration-response curves to angiotensin II (AII) of rat small mesenteric resistance arteries with functional endothelium taken from WKY (a) or SHR (b). The values are mean of 5 experiments, in the absence (○) or presence of L-NAME (10^{-4}M; ■), indomethacin (10^{-5}M; ●) and L-NAME plus indomethacin (◆). Vertical lines show s.e. mean.

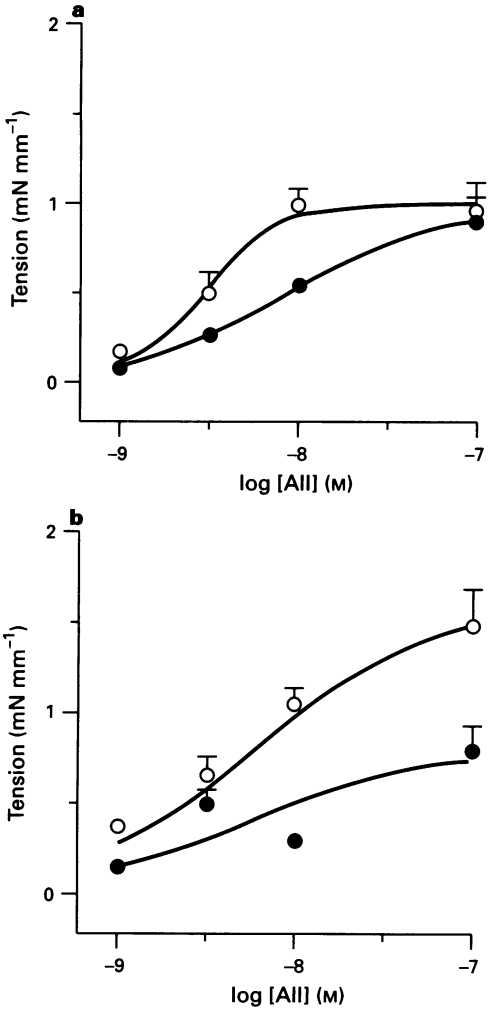

Figure 5 Effect of GR32191 B on concentration-response curves to angiotensin II (AII) of rat small mesenteric resistance arteries with functional endothelium taken from WKY (a) or SHR (b). The values are mean of 6 or 5 experiments, in the absence (○) or in the presence (●) of GR32191 B (3×10^{-6} M), respectively; vertical lines show s.e. mean.

Discussion

The results presented suggest that both endothelium-dependent and endothelium-independent synthesis of COX vasoconstrictor products are involved in contractile responses elicited by AII in small mesenteric arteries from SHR, whereas only endothelium-derived COX products are involved in WKY rat arteries. They suggest that these COX products are different according to whether they are synthesized in the endothelium or in smooth muscle cells, as their effect was differentially inhibited by the TP-receptor antagonist GR32191 B. In addition, it was found that the inhibitory effects of indomethacin and GR32191 B on AII-induced contractions were larger in SHR than in WKY arteries. However, the increased inhibitory effect of indomethacin was blunted by an enhanced potentiating effect of L-NAME in SHR. The latter finding suggests that an increased endothelium-derived NO had the effect of neutralizing the enhanced synthesis of vasoconstrictor COX products in SHR arteries.

Vasoconstriction produced by AII was increased in SHR arteries without endothelium and in SHR arteries with endothelium exposed to L-NAME. This increase in absolute tension does not necessarily imply alterations in receptors or signalling pathways, since greater contractility of resistance arteries has been associated with decreased wall/lumen ratio in the SHR (Mulvany & Halpern, 1977). However, the differential effects of various drugs distinguished the relative participation of COX products and endothelium-derived NO in the two strains.

In the absence of functional endothelium, both indomethacin and GR32191 B had no significant effect in WKY arteries, but indomethacin markedly attenuated contractile responses to AII in SHR arteries. This suggests that COX products are involved in the response to AII of SHR, but not WKY endothelium-denuded vessels. The failure of the TP-receptor antagonist GR32191 B (Lumley *et al.*, 1989) to mimic the effect of indomethacin in these conditions indicates that one or more COX products different from the endogenous agonists of the TP-receptor are involved. Further studies are needed to determine the nature of this factor. Vasoconstrictor eicosanoids like prostaglandin E_2 and prostaglandin $F_{2\alpha}$ might be involved (for review, see Coleman *et al.*, 1994).

The results obtained in arteries with functional endothelium show that contractile responses elicited by AII in these conditions involve a major contribution of COX products in both strains, as contractions were almost completely abolished by indomethacin. In addition, they indicate that these products partially act via TP-receptors, as contractions were partially inhibited by GR32191 B. Comparison with the results obtained in endothelium-denuded arteries showed that the GR32191 B-sensitive component is endothelium-dependent. Finally, a comparison between SHR and WKY vessels showed that the GR32191 B-sensitive component of the contraction is markedly enhanced in SHR. Altogether these results support the view that endothelium-dependent production of one or several thromboxane A_2 (TXA_2) or prostaglandin H_2 (PGH_2) like compounds active at TP-receptors is involved in AII-induced contraction of small mesenteric arteries from both SHR and WKY rats, although this mechanism is increased in the SHR. The present findings are consistent with data from a previous study showing that AII infusion releases TXA_2 in rats and that TXA_2 accounts for a large part of the short term pressor response to AII (Wilcox & Welch, 1990). Whether the endothelial production of a vasoconstrictor prostaglandin or its effect was enhanced in AII exposed mesenteric resistance arteries from the SHR remains to be determined. The effects of AII and TXA_2 have previously been found to be exaggerated in renal vessels from SHR (Chatziantoniou *et al.*, 1990; Chatziantoniou & Arendshorst, 1991).

Independent of the presence or absence of functional endothelium, losartan was able to abolish contractile responses induced by AII in arteries of either strain. This indicates that the responses were produced via AT_1 receptors. In superior mesenteric artery of the rat, Chen *et al.* (1995) found that the contractile response to AII involved the activation of AT_1 receptors located on both smooth muscle and endothelial cells. In other vessels such as the rat carotid artery, activation of endothelial AT_1 receptors produces NO-mediated relaxation rather than contraction (Boulanger *et al.*, 1995). Recently, it was found that AII failed to enhance endothelial NO production in small mesenteric arteries from Wistar rats (Andriantsitohaina *et al.*, 1996). Thus, the endothelium contributes to the differential vasoactive properties of AII in blood vessels. It is well established that in SHR, ACh induces endothelium-dependent vasoconstriction via the release of a vasoconstrictor prostaglandin, possibly TXA_2/PGH_2 (Ito *et al.*, 1991; Dai *et al.*, 1992; Lang *et al.*, 1995), and it was verified that this dual effect of ACh (relaxant at low concentration and constrictor at high concentration) was present in SHR vessels used here. The results obtained in AII exposed vessels indicate that both endothelium-derived NO (as shown by the experiments with L-NAME) and endothelium-derived TXA_2/PGH_2 (as shown by the experiments with GR32191 B and indomethacin) release were enhanced in SHR vessels. However, the results obtained in this study do not permit us to distinguish whether the increased release of these factors is due to an enhancement of basal release or to stimulation by AII.

Despite its ability to produce a vasoconstrictor prostaglandin, the presence of endothelium caused pronounced attenuation of AII elicited contractions, and this effect of the endothelium was enhanced in SHR. L-NAME markedly reduced the inhibitory influence of the endothelium in both strains. Furthermore, its effect was larger in SHR than in WKY arteries. Thus, these results support the view that increased NO release counteracts the increased release of a vasoconstrictor factor by the endothelium in SHR small mesenteric arteries exposed to AII; in contrast with the previously obtained impairment of NO release by endothelial cells from SHR (Malinsky *et al.*, 1993). However, in an earlier study we also found that an increased relaxing influence of the endothelium counteracted the enhanced contractile responses to neuropeptide Y in the rat mesenteric artery from SHR (Andriantsitohaina *et al.*, 1991). Differential regulation of endothelial NO release may depend on the agonist producing endothelium-dependent relaxation, like ACh, or contraction, like AII and neuropeptide Y. The result of enhancing the release of both relaxing and contracting factors by the endothelium of SHR was that the contraction elicited by AII did not differ in small mesenteric arteries with endothelium from either SHR or WKY. This may partially explain the recently described dissociation between enhanced production of endothelial vasoconstrictor prostaglandin in isolated vessels and increased blood pressure in SHR (Tesfamariam & Ogletree, 1995).

Another finding in our study was that, in either strain, the responses to AII were not significantly different in arteries without functional endothelium and in arteries with endothelium exposed to L-NAME, suggesting that NO accounts for the major part of the relaxing effect of endothelium in these experimental conditions. It is worth mentioning that the experiments were performed in the presence of KCl (25 mM) in the medium. In these conditions, the effect of a hyperpolarizing factor, possibly released by endothelium, was blunted, as previously observed (Andriantsitohaina *et al.*, 1996). However, a recent study suggested the existence of a novel endothelium-derived relaxing factor (EDRF) in the endothelium of rat mesenteric arterial bed, which acts through increasing the level of adenosine 3′: 5′-cyclic monophosphate (Kamata *et al.*, 1996). This novel EDRF might contribute, together with NO, to the attenuation of the effect of the endothelial COX contractile product(s) observed in vessels from SHR. Further studies are required for a more complete understanding of the complex interactions between the different relaxing and contractile endothelial factors.

In conclusion, evidence was obtained to support a role for COX products in the contractile responses of rat mesenteric resistance arteries to AII and in their alterations in the SHR. A vasoconstrictor prostaglandin of endothelial origin, probably TXA_2, may activate TP receptors in either strain. Its contribution to contraction elicited by activation of AII AT_1 receptors is increased in arteries from SHR. In addition, another COX product of extra-endothelial origin, which does not activate TP receptors, appears to be involved in the responses of SHR arteries to AII. However, enhanced contraction induced by these COX products is counteracted by an enhanced relaxation produced by endothelium-derived NO, in SHR arteries exposed to AII.

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