Cysteinyl leukotrienes mediate enhanced vasoconstriction to angiotensin II but not endothelin-1 in SHR

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Shastri, Shailesh, J. Robert McNeill, Thomas W. Wilson, Ramarao Poduri, Chamanlal Kaul, and Venkat Gopalakrishnan. Cysteinyl leukotrienes mediate enhanced vasoconstriction to angiotensin II but not endothelin-1 in SHR. Am J Physiol Heart Circ Physiol 281: H342–H349, 2001.—We assessed whether cysteinyl leukotrienes mediate the vasoconstrictor responses to angiotensin II and endothelin-1 in the mesenteric vascular bed of Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) perfused ex vivo at a constant flow rate of 5 ml/min with Krebs buffer. Maximal perfusion pressure response (Emax) but not EC50 values to angiotensin II (P < 0.001) and endothelin-1 (P < 0.01) were significantly higher in the SHR, whereas the responses to potassium chloride remained unchanged. Inclusion of the selective 5-lipoxygenase inhibitor AA-861 or the cysteinyl leukotriene receptor antagonist MK-571 significantly reduced the vasoconstrictor responses to angiotensin II but not to endothelin-1 and potassium chloride. The reduction in Emax to angiotensin II was more pronounced in SHR (P < 0.001) than in WKY (P < 0.05) rats. Cysteinyl leukotrienes LTC4, LTD4, and LTE4 (1 μM)-evoked vasoconstrictor responses were significantly higher in SHR (P < 0.05), whereas LTB4 failed to evoke any response in either strain. These data suggest that 5-lipoxygenase metabolites, particularly cysteinyl leukotrienes, contribute to the exaggerated vasoconstrictor responses to angiotensin II but not to endothelin-1.

12-LO activity has been noted in SHR, and inhibition of 12-LO activity reduced blood pressure in renovascular hypertensive rats (21, 27). Despite such evidence implicating a role for 12-LO products in experimental models of hypertension, no systematic studies have been undertaken so far to examine the contribution of 5-LO-derived products in ANG II-evoked responses. 5-LO activation leads to generation of leukotriene B4 (LTB4) and cysteinyl leukotrienes such as LTC4, LTD4, and LTE4. Among these, LTC4 and LTD4 have been shown to increase the tone of mesenteric arteries (7, 28). Moreover, LTD4-evoked pressor responses were shown to be higher in SHR (36). Recent studies (9, 20, 23, 24) also demonstrated that leukotrienes are synthesized and generated from vascular endothelial cells and VSM cells. Therefore, the present study was undertaken to assess the role of leukotrienes in ANG II-evoked vasoconstrictor responses ex vivo in the perfused mesenteric vascular bed (MVB) isolated from Wistar-Kyoto (WKY) rats and SHR. It is well known that this preparation contributes to the resistance function of circulation (5).

Previously, we and others (2, 4, 6) demonstrated that ANG II-evoked vasoconstrictor/vasopressor responses are partly dependent on endothelin-1 (ET-1), particularly in the MVB. Moreover, ET-1, the most potent and efficacious agonist, is also known to stimulate arachidonic acid (AA) production and consequent release of several of its metabolites in various tissues. In the kidney, the tubular effects of ET-1 were reported to be partly dependent on endothelin-1 (21, 27). Despite such evidence implicating a role for 12-LO products in experimental models of hypertension, no systematic studies have been undertaken so far to examine the contribution of 5-LO-derived products in ANG II-evoked responses. 5-LO activation leads to generation of leukotriene B4 (LTB4) and cysteinyl leukotrienes such as LTC4, LTD4, and LTE4. Among these, LTC4 and LTD4 have been shown to increase the tone of mesenteric arteries (7, 28). Moreover, LTD4-evoked pressor responses were shown to be higher in SHR (36). Recent studies (9, 20, 23, 24) also demonstrated that leukotrienes are synthesized and generated from vascular endothelial cells and VSM cells. Therefore, the present study was undertaken to assess the role of leukotrienes in ANG II-evoked vasoconstrictor responses ex vivo in the perfused mesenteric vascular bed (MVB) isolated from Wistar-Kyoto (WKY) rats and SHR. It is well known that this preparation contributes to the resistance function of circulation (5).

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the cysteinyl leukotriene (cysLT1) receptor antagonist (E)-3-[[3-[2-(7-chloro-2-quinoliny1)ethenyl]phenyl][3-dimethylamino]-3-oxopropyl]thio)methyl]thio)-propionic acid, sodium salt (MK-571) (12, 35).

MATERIALS AND METHODS

Perfused mesenteric vascular bed preparation. Experiments were performed using 16-wk-old male WKY and SHR (Charles River, St. Constant, Quebec, Canada). The baseline systolic blood pressure (determined by tail cuff) was 115 ± 3 and 167 ± 5 mmHg in WKY and SHR groups, respectively. All procedures were conducted in accordance with the guidelines of the University Animal Care Committee. Animals were euthanized under pentobarbital anesthesia (50 mg/kg ip), after which the superior mesenteric artery was cannulated (14). The MVB was isolated (14), after which the superior mesenteric artery was cannulated (14). The MVB was perfused with either AA-861 or MK-571 for a period of 30 min before the addition of ANG II, ET-1, or KCl. A Grass polygraph (Quincy, MA) then electronically integrated the pulsatile pressure signal as an increase in perfusion pressure (in mmHg).

Experimental protocol. An equilibration period of 1 h was allowed to stabilize the MVB baseline perfusion pressure. This was followed by a single concentration of phenylephrine (PE, 70 μM) perfusion for 30 min. A bolus dose of acetylcholine (ACh, 10 μM) was injected to assess the functional integrity of the endothelium (14, 33). Vasodilatation in response to ACh was taken as acceptance criteria to confirm the functional integrity of the endothelium. The preparation was then subsequently washed with buffer to allow it to recover to baseline. Constrictor responses to increasing concentrations of KCl (20–80 mM) were determined after adjustment for isotonicity of the buffer solution by equivalent reduction in NaCl. After repeated washings, each concentration of ANG II (1, 10, 100 nM and 1 and 10 μM) was injected as a bolus infusion, and increases in perfusion pressure for each concentration were recorded. At concentrations >1 μM, ANG II-evoked maximal increases in perfusion pressure were always lower than the effect seen at 1 μM. Infusion of a higher concentration of ANG II was given only after the tissue had recovered from the constrictor response to a previous concentration of ANG II and after the baseline perfusion pressure was attained. Concentration-response (C-R) curves to ANG II were determined only once in each MVB preparation. Responses to increasing concentrations of ET-1 (100 pM–1 μM) were performed in a cumulative manner in separate preparations to avoid cross peptide desensitization. Studies with 5-LO inhibitor AA-861 (at both 10 and 30 μM concentrations) or cysLT1 receptor antagonist MK-571 (10 μM) were conducted in a parallel fashion. The responses to each agonist in either the presence or the absence of either AA-861 or MK-571 were determined in a minimum of at least five separate MVB preparations of WKY and SHR strains. The MVB was perfused with either AA-861 or MK-571 for a period of 30 min before the addition of ANG II, ET-1, or KCl. The preparations that did not receive AA-861 or MK-571 performed served as controls. In preliminary experiments, varying concentrations of either AA-861 (1–100 μM) or MK-571 (1–30 μM) were employed. The maximal inhibitory effects with AA-861 and MK-571 were reached at 30 and 10 μM, respectively. In another set of experiments, cumulative C-R curves to leukotrienes (LTB4, LTC4, LTD4, and LTE4, 100 pM–1 μM) were determined to assess their vasoconstrictor efficacies. The vasoconstrictor responses to cysteinyl leukotrienes were very low in the MVB preparations when endogenous leukotriene generation was not blocked using AA-861, the 5-LO inhibitor. Hence, the C-R curves to cysteinyl leukotrienes were always determined in the presence of AA-861 (30 μM). The effect of MK-571 was studied on LTD4-mediated responses to confirm the stimulation of the cysLT1 receptor subtype in leukotriene-mediated vasoconstriction.

Reagents. Angiotensin II (human ANG II) and endothelin-1 (ET-1: human, porcine, canine rat, and mouse) were purchased from Bachem (Torrance, CA). PE, ACh, and AA-861 were purchased from Sigma Chemical (Oakville, Ontario, Canada). Propronic acid, MK-571, and leukotrienes (LTB4, LTC4, LTD4, and LTE4) were obtained from Cayman Chemicals (Ann Arbor, MI). Krebs solution salts were of analytic grade obtained from BDH (Toronto, Ontario, Canada).

Analysis of responses. C-R curves were analyzed individually for the estimation of EC50 (expressed as negative logarithm of the concentration required to produce 50% of the maximal response) and maximal perfusion pressure response (Bmax in mmHg) values. Repeated measures ANOVA were used to assess the differences in the responses to agonists and KCl for different preparations. The experimental values are expressed as means ± SE. Comparison of mean values between various groups was performed by ANOVA (Super Anova Package). Simultaneous multiple comparisons were examined by Scheffe’s F-test.

RESULTS

Comparison of responses to ANG II, ET-1, and KCl in MVB of WKY and SHR strains. After 1 h of equilibration, the mean baseline perfusion pressure was 27 ± 2 and 32 ± 3 mmHg in WKY and SHR groups, respectively. The differences in mean values were not statistically significant. Both agonists (ANG II and ET-1), as well as depolarization with KCl, evoked concentration-dependent increases in perfusion pressure in the MVB of WKY and SHR strains with the following rank-order potency (negative log molar EC50): ET-1 > ANG II > KCl. No significant differences in EC50 values for either ANG II or ET-1 were noted between WKY and SHR preparations. The rank order of efficacy was ET-1 > KCl > ANG II in both groups. ANG II efficacy was significantly lower when expressed as a percentage of Emax to ET-1 (12 ± 1%). The Emax values for both ANG II (P < 0.001) and ET-1 (P < 0.01) were significantly higher in the SHR compared with WKY group, whereas both EC50 and Emax values for KCl were similar between WKY and SHR groups (Table 1). ANG II responses when normalized with respect to the percent Emax to KCl were 27 ± 4% in WKY versus 49 ± 3% in SHR (P < 0.001). Because the normalized data did not differ from the absolute values of increases in perfusion pressure calculated for ANG II, these values are shown in Table 1.
LEUKOTRIENES MEDIATE VASOCONSTRICTOR RESPONSE TO ANG II

Table 1. Analyses of concentration-response curves to ANG II, ET-1, and KCl perfusion in either presence or absence of AA-861 or MK-571 in perfused MVB ex vivo isolated from 16-wk-old male WKY and SHR strains

<table>
<thead>
<tr>
<th>Agonist</th>
<th>WKY (EC50, mmHg)</th>
<th>SHR (EC50, mmHg)</th>
<th>WKY (Emax, mmHg)</th>
<th>SHR (Emax, mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANG II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.70 ± 0.20</td>
<td>8.20 ± 0.20</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>AA-861 (10 μM)</td>
<td>7.50 ± 0.50</td>
<td>8.1 ± 1</td>
<td>7.24 ± 0.20</td>
<td>7.35 ± 0.20</td>
</tr>
<tr>
<td>AA-861 (30 μM)</td>
<td>7.80 ± 0.29</td>
<td>6.1 ± 1</td>
<td>7.15 ± 0.50</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>MK-571 (10 μM)</td>
<td>7.70 ± 0.33</td>
<td>7 ± 1</td>
<td>7.50 ± 0.08</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>ET-1</td>
<td>8.10 ± 0.03</td>
<td>170 ± 10</td>
<td>8.00 ± 0.05</td>
<td>210 ± 3</td>
</tr>
<tr>
<td>AA-861 (30 μM)</td>
<td>8.03 ± 0.15</td>
<td>169 ± 11</td>
<td>7.91 ± 0.15</td>
<td>210 ± 3</td>
</tr>
<tr>
<td>MK-571 (10 μM)</td>
<td>7.95 ± 0.12</td>
<td>178 ± 5</td>
<td>7.90 ± 0.01</td>
<td>215 ± 4</td>
</tr>
<tr>
<td>KCl</td>
<td>1.48 ± 0.01</td>
<td>44 ± 2</td>
<td>1.43 ± 0.04</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>AA-861 (30 μM)</td>
<td>1.51 ± 0.01</td>
<td>47 ± 5</td>
<td>1.48 ± 0.03</td>
<td>51 ± 5</td>
</tr>
<tr>
<td>MK-571 (10 μM)</td>
<td>1.50 ± 0.01</td>
<td>48 ± 3</td>
<td>1.45 ± 0.03</td>
<td>50 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 separate experiments. Response to each agonist was determined only once in each mesenteric vascular bed (MVB) preparation. Either AA-861 (5-lipoxygenase inhibitor) or MK-571 (leukotriene antagonist) were maintained in perfusion medium for a period of 30 min before and during perfusion with increasing concentrations of each agonist. Emax, maximal elastance; WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rat; ET-1, endothelin-1. *P < 0.05 compared with respective control group; †P < 0.001 compared with respective control group; §P < 0.001 compared with respective WKY group.

Attenuation of vasoconstrictor responses to ANG II but not ET-1 by AA-861 and MK-571. Inclusion of either 5-LO inhibitor AA-861 (10 and 30 μM) or cysteinyI leukotriene (cysLT1) receptor antagonist MK-571 (10 μM) in the perfusion buffer failed to affect the basal tone of the MVB of WKY and SHR strains. AA-861 (10 and 30 μM) reduced the Emax to ANG II in both WKY (P < 0.05) and SHR (P < 0.001) preparations (Table 1, Fig. 1). The maximal inhibition of ANG II (1 μM) response was 34 ± 2% in WKY and 50 ± 5% in SHR (P < 0.05) and 50 ± 2% in WKY and 67 ± 7% in SHR (P < 0.05) at 10 and 30 μM concentrations of AA-861, respectively. Thus the attenuating effect of AA-861 was concentration dependent; moreover, it was more pronounced in the MVB of SHR. Importantly, the significant difference in the Emax values for ANG II seen between WKY (12 ± 1 mmHg) and SHR (24 ± 1 mmHg; P < 0.001) preparations was abolished when AA-861 (30 μM) was present in the perfusate (Emax 6 ± 1 mmHg in WKY vs. 8 ± 1 mmHg in SHR). Strikingly, AA-861 failed to inhibit the responses to ET-1 (Table 1, Fig. 2) and KCl (Table 1, Fig. 3). These data suggest that inhibition of 5-LO-mediated events selectively reduced the exaggerated vasoconstrictor responses to ANG II. The cysteinyI leukotriene receptor antagonist MK-571 significantly inhibited ANG II-mediated vasoconstriction in WKY (P < 0.05) and SHR (P < 0.001) strains (Fig. 1). The inhibitory effect was higher in SHR (maximal inhibition 42 ± 3% in WKY).
and 54 ± 4% in SHR, P < 0.05). Responses to both ET-1 and KCl remained unaffected in the presence of MK-571, despite their higher levels of efficacy (Figs. 2 and 3).

Vasoconstrictor responses to leukotrienes in the MVB of WKY and SHR. Whereas LTB4 failed to elicit any vasoconstrictor response (up to 1 μM concentration) in either WKY or SHR preparations, LTC4, LTD4, and LTE4 induced concentration-dependent increases in perfusion pressure in the MVB of both strains in the presence of AA-861 (Fig. 4, A–C). The responses to these agonists reached their Emax at 100 nM concentration in MVB preparations of WKY strain, and the effect reached a plateau at 1 μM concentration with the following rank order of efficacy: LTD4 > LTC4 > LTE4. In contrast, the C-R curves were linear and failed to plateau in the MVB of SHR. The vasoconstrictor responses to LTC4, LTD4, and LTE4 (at 1 μM) were significantly higher (P < 0.05) in SHR compared with WKY preparations (Fig. 4, A–C). The inclusion of cysLT1 antagonist MK-571 significantly inhibited LTD4-mediated increases in perfusion pressure in both WKY and SHR preparations (Fig. 5).

DISCUSSION

First, and most importantly, the present study demonstrates for the first time a role for the 5-LO pathway in mediating vasoconstrictor responses to ANG II in the MVB. Second, we demonstrate that cysLT1 receptor antagonist MK-571 reduced the vasoconstrictor responses to ANG II in the MVB of both SHR and WKY strains. These key observations suggest that ANG II could promote 5-LO-mediated cysteinyl leukotriene generation that contributes to the vasoconstrictor responses in the MVB of these strains. Third, both AA-861 and MK-571 selectively reduced the vasoconstrictor responses to ANG II but not to ET-1. The inhibitory effects were attained despite the much lower efficacy of ANG II in this preparation. Fourth, we show that the vasoconstrictor responses to ANG II and ET-1 but not depolarizing response to KCl were significantly higher in the MVB of SHR. Finally, cysLT1 receptor antagonist reduced the exaggerated vasoconstrictor responses to ANG II (but not to ET-1) in the MVB of SHR, which suggests that the overactive leukotriene generation could contribute to increased vasoconstriction in the MVB and hypertension in SHR. The significance of these key observations is discussed below.

Exaggerated responses to ANG II and ET-1 in hypertension. Emax values for both ANG II and ET-1 were significantly higher in the MVB of SHR compared with WKY rats. This is consistent with previous reports that have demonstrated higher efficacy for ANG II and ET-1 in the perfused MVB of SHR (15, 33). Thus the exaggerated vascular response to ANG II and ET-1 but not to KCl confirms agonist-specific modulation of vascular responses in SHR. Because MVB contributes to the resistance function of circulation, the exaggerated responses arising from this preparation could contribute to hypertension (5).

Selective attenuation of ANG II vasoconstriction by 5-LO inhibitor AA-861. Region-specific LO converts AA to promote the formation of 5, 12, and 15-hydroperoxyeicosatetraenoic acid (5-HPETE), which are subsequently converted to corresponding hydroxyeicosatetraenoic acid (HETEs). 5-HETE is further metabolized to...
various leukotrienes. The nonspecific LO inhibitors phenidone and baicalein have been shown to reduce the vasoconstrictor responses of the femoral artery in vitro and the pressor responses in vivo to ANG II in Sprague-Dawley rats (31). Moreover, the potent antihypertensive property of phenidone has been demonstrated in renovascular hypertensive rats and in the SHR model (21, 32). Studies have also shown enhanced level of 12-LO activity in ANG II-dependent forms of experimental hypertension (21, 27). Thus the important contribution of 12-LO pathway in hypertension in these rat models is well established. The present study demonstrates that in addition to 12-LO, 5-LO-derived products may also play an important contributory role in the vasoconstrictor responses to ANG II, particularly in the SHR model. Because the level of inhibition of ANG II-evoked constrictor responses by AA-861 was much more pronounced in SHR, it is possible that the 5-LO pathway may account for exaggerated responses to ANG II in SHR. Several studies (10, 13, 21, 25, 27) have demonstrated that there is increased generation of eicosanoids in various disease conditions such as ischemic injury, hypertension, and diabetes. Interestingly, increased generation of LO-derived AA mediators has been noted in the mesenteric vasculature of the SHR strain (8). The data from the present study using a selective 5-LO blocker (AA-861) provide evidence that among the various eicosanoids, 5-LO-derived products could play an important role in the exaggerated vasoconstrictor responses to ANG II in the SHR strain. AA-861 was found to be more selective for 5-LO inhibition in a number of test systems. Moreover, the IC$_{50}$ value for AA-861 to inhibit 12-LO was two orders of magnitude higher than the concentration required to block 5-LO (35). At a concentration of 10 μM, AA-861 does not inhibit the 12-LO activity. In the present study, we demonstrate that AA-861 (10 μM) significantly inhibited ANG II-induced vasoconstrictor responses in both WKY and SHR. However, the maximal inhibitory effect of AA-861 was reached at a concentration of 30 μM. AA-861 (30 μM) did not inhibit 12-LO in bovine platelets (35). Therefore, it is reasonable to predict that inhibition of 5-LO rather than 12-LO mainly accounts for the attenuation of ANG II-evoked vasoconstriction by AA-861. In contrast to blockade of ANG II responses, perfusion with AA-861 failed to inhibit the vasoconstrictor responses to ET-1, although previous studies by others have demonstrated that responses to ET-1 in the lung, kidney, and VSM cells are dependent on 5-, 12-, and 15-LO-derived products (16, 22, 26). Failure of AA-861 to inhibit the responses to ET-1 in the MVB of both WKY and SHR confirms that ET-1 may recruit mechanisms other than 5-LO-derived mediators for its evoked vasoconstrictor responses in this preparation.

**Vasoconstrictor responses to leukotrienes in MVB of WKY and SHR.** The activation of the 5-LO pathway leads to the formation of LTB$_4$ and cysteinyl leukotrienes such as LTC$_4$, LTD$_4$, and LTE$_4$. LTC$_4$ and LTD$_4$ exert constrictor responses in various blood vessels,
including rat mesenteric artery (7, 28). In the present study, we noticed that LTB4 did not evoke a constrictor response in the MVB of both SHR and WKY strains (data not shown). This is in agreement with the previous report that demonstrated the agonistic activity of other leukotrienes but not LTB4 in the human internal mammary artery and saphenous vein (1). Whereas LTC4 and LTE4 induced a weaker response in MVB, LTD4 evoked much larger responses in this preparation. LTC4-, LTD4-, and LTE4-induced vasoconstrictor responses were also significantly higher in the MVB of SHR (Fig. 4). LTD4 evoked a pressor response that was followed by a depressor phase in SHR, and this was attributed to regional variations in the vascular responses to LTD4 in SHR strain (3, 36). However, during both pressor and depressor phases, the blood flow to the splanchnic region was drastically reduced, confirming a powerful vasoconstrictor response in this vascular region. More importantly, this study also concluded that the constrictor responses to LTD4 arising from the splanchnic vascular region was significantly higher and that it contributed to the enhanced pressor responses to LTD4 in SHR (36). Our data using an isolated perfused MVB preparation ex vivo supports the observations reported by the hemodynamic study. These results support the notion that cysteinyl leukotrienes are the 5-LO-derived products in mesenteric vasculature that may contribute to vasoconstrictor responses to ANG II. Cysteinyl leukotrienes activate at least two receptors, designated as cysLT1 and cysLT2, located on both endothelial cells as well as VSM cells (11, 12). The complex interaction of leukotrienes with their receptor subtypes are known to promote Ca2+ mobilization in both endothelial cells and VSM cells contributing to both vasodilatation as well as vasoconstriction (12, 36). The blockade of vasoconstrictor re-

Fig. 4. Line graphs show C-R curves to increasing concentrations of leukotrienes LTC4 (A), LTD4 (B), and LTE4 (C) infusion in the presence of AA-861 (30 μM) in the perfused MVB ex vivo isolated from WKY and SHR strains. *P < 0.05 compared with respective data point in WKY group.

Fig. 5. Line graphs showing effect of MK-571 (10 μM) on LTD4-evoked vasoconstrictor responses in the MVB of WKY (A) and SHR (B) strains. MK-571 was perfused 30 min before and during the addition of serially increasing concentrations of LTD4. *P < 0.05 and **P < 0.01 compared with respective data points for the control group.
sponses to LTD₄ by MK-571 suggests that cysteinyll leukotrienes mediate vasoconstrictor responses in MVB of SHR and WKY via activation of cysLT₁ receptor subtype. From the prohibitive costs, we have not examined the responses to high concentrations (>1 μM) of LTD₄ in the perfused MVB in either the presence or absence of MK-571; however, others (12) have demonstrated that MK-571 exerts competitive antagonism of LTD₄-evoked responses.

**Inhibition of ANG II vasoconstriction by cysteinyl leukotriene antagonist.** Inhibition of ANG II vasoconstriction by cysLT₁ receptor antagonist MK-571 provides further evidence in support of involvement of cysteinyll leukotrienes in ANG II-evoked vasoconstrictor responses. cysLT₁ receptor is known to signal through elevation in cytosolic free calcium (12). Thus leukotrienes may mediate ANG II-evoked vasoconstriction via cysLT₁ receptor activation linked to calcium mobilization. Recently, ANG II-induced vasoconstrictor responses of the rat pulmonary artery and human internal mammary artery were shown to be dependent on cysteinyll leukotrienes (29, 34). There is considerable evidence to support that leukotrienes are generated from vascular endothelial cells as well as from VSM cells (9, 20, 23, 24). The present study, however, did not examine the source and origin of leukotriene production (endothelial cells vs. VSM cells) subsequent to ANG II receptor activation. This is because the vasoconstrictor responses to ANG II were much weaker (10–12 mmHg) and endothelium-denudation led to further loss of responses to ANG II. We and others (2, 4, 6) have previously demonstrated that ANG II-evoked responses are dependent on endogenous ET-1, and this may contribute to decreased responses in endothelium-denuded perfused MVB preparations. Therefore, such practical difficulties of much lower responses encountered in endothelium-denuded MVB preparations did not permit us to assess the effects of either AA-861 or MK-571 on ANG II-evoked responses in the absence of endothelium.

While this paper was under revision, Stanke-Labesque et al. (30) reported a similar finding implicating the involvement of 5-LO and cysteinyll leukotrienes in ANG II-evoked vasoconstrictor responses in the aorta of SHR. The present study identifies the existence of this important mechanism in a more relevant preparation of MVB that contributes to the resistance function of the circulation (5) rather than the aorta, which is a conduit-type blood vessel that contributes minimally to the resistance function and hypertension.

In conclusion, the present study demonstrates the involvement of cysteinyll leukotrienes as 5-LO pathway mediators in the vasoconstrictor responses to ANG II in the MVB. The pronounced attenuation of ANG II-evoked vasoconstriction by both 5-LO inhibitor AA-861 and cysLT₁ receptor antagonist MK-571, as well as the observation that the constrictor responses to cysteinyll leukotrienes are exaggerated in the perfused MVB of SHR, support the concept that 5-LO pathway may contribute to hypertension in this model. Therefore, it may be worthwhile to consider 5-LO inhibitors and cysLT₁ antagonist as an alternate approach to overcome exaggerated vasoconstrictor responses and associated hypertension in SHR.

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