Cysteinyl Leukotrienes Modulate Angiotensin II Constrictor Effects on Aortas From Streptozotocin-Induced Diabetic Rats

Gaëlle Hardy, Françoise Stanke-Labesque, Michel Peoc’h, Ahmed Hakim, Philippe Devillier, Françoise Caron, Sandrine Morel, Patrice Faure, Serge Halimi, Germain Bessard

Abstract—Angiotensin II (Ang II) is a vasopressor peptide involved in the pathogenesis of cardiovascular diseases associated with diabetes mellitus. We have previously reported that the 5-lipoxygenase–derived products, particularly the cysteinyl leukotrienes (CysLTs), are involved in Ang II–induced contraction. In this study, we demonstrated that CysLTs contribute to the contraction elicited by Ang II in isolated aortas from streptozotocin-induced diabetic (SS) rats but not from insulin-treated diabetic rats, fructose-fed rats, or control rats. In an organ bath, pretreatment with the 5-lipoxygenase inhibitor (AA861, 10 μmol/L) reduced by 37.6±8.2% and 30.1±10.9% the Ang II–induced contractions in intact and endothelium-denuded aortic rings, respectively, from SS rats. In contrast, the CysLT1 receptor antagonist (MK571, 1 μmol/L) or the dual CysLT1/CysLT2 receptor antagonist (BAY-u9773, 0.1 μmol/L) did not affect Ang II–induced contraction. In addition, Ang II induced a 6.2±1.5-fold increase in CysLT release through the stimulation of the Ang II type 1 receptor. Furthermore, the urinary excretion of leukotriene E4 was increased in SS rats (leukotriene E4, 13.7±2.9 ng/24 h [SS rats, n=10] versus 1.5±0.5 ng/24 h [control rats, n=6]; P<0.0004). These data suggest the activation of the 5-lipoxygenase pathway in SS rats, and the involvement of 5-lipoxygenase–derived products, particularly the CysLTs, in Ang II–induced contraction in aortas from SS rats through stimulation of CysLT receptors different from the well-characterized CysLT1 or CysLT2 receptor. (Arterioscler Thromb Vasc Biol. 2001;21:1751-1758.)

Key Words: angiotensin II ▪ cysteinyl leukotrienes ▪ diabetes ▪ insulin ▪ rat aortas

Cardiovascular complications are a major cause of morbidity and mortality in type I and II diabetes mellitus. However, the etiology of the increased susceptibility to cardiovascular diseases in diabetics still remains poorly understood. Angiotensin II (Ang II) is a vasoactive peptide that has been suggested to be involved in the pathogenesis of cardiovascular diseases. In this regard, an increased serum ACE activity has been reported in diabetic subjects with vascular complications and in streptozotocin-induced diabetic rats. Furthermore, the vascular reactivity to Ang II is increased in alloxan-induced diabetic rats, and the vasopressor effects of Ang II are enhanced in type I (insulin-dependent) diabetic patients.

Compelling evidence suggests that lipoxygenase-derived eicosanoids contribute to the constrictor effects of Ang II. Lipoxygenases catalyze the formation of 5-, 12-, and 15-hydroperoxyeicosatetraenoic acids (HPETEs), which are subsequently transformed to the corresponding hydroxyeicosatetraenoic acids (HETEs) and, in the case of 5-HPETE, to leukotriene B4 (LTB4) or cysteinyl leukotrienes (CysLTs: LTC4, LTD4, and LTE4). Nonselective lipoxygenase inhibition has been reported to reduce the in vitro contraction elicited by Ang II in rat femoral arteries. In addition, specific inhibition of leukotriene biosynthesis by blockade of 5-lipoxygenase–activating protein reduced the Ang II pulmonary pressor effect in hypoxic rats. Moreover, it has been recently reported that leukotriene biosynthesis inhibition or CysLT1 receptor blockade reduced Ang II–mediated contractions in human internal mammary arteries and in aortas and mesenteric arteries from spontaneously hypertensive rats. Taken together, these data suggested the involvement of 5-lipoxygenase–derived products, and particularly CysLTs, in Ang II–mediated contractile effects.

Furthermore, in diabetes, vascular complications have been linked to abnormalities in the arachidonic acid cascade to either the cyclooxygenase or the lipoxygenase pathways. In this regard, endothelial and smooth muscle cells cultured under hyperglycemic conditions produced increased amounts of HETEs. In addition, polymorphonuclear leukocytes from diabetics displayed an increased release of lipoxygenase-derived eicosanoids. Among the lipoxygenase-derived products, the CysLTs have been shown to induce enhanced coronary constrictor responses in diabetic rats compared with nondiabetic rats.
In this context, the present study was designed to assess the involvement of the 5-lipoxygenase metabolites, and particularly the CysLTs, in the vasoconstrictor effects of Ang II on isolated aortas from streptozotocin-induced diabetic and fructose-fed rats. Because insulin has been shown to inhibit 12-HETE production on human cultured glomerular cells, the influence of insulin treatment on CysLT production and Ang II vasoconstrictor effects was also investigated.

Methods

The care and use of animals in the present study were in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (publication No. [NIH] 85-23, revised 1985).

Experimental Groups

Streptozotocin-Induced Diabetic Rats

Experiments were conducted on adult male Wistar rats (weight range 220 to 240 g) from IFFA CREDO (France). Rats were randomly assigned to 3 groups. Two groups were made diabetic by a single injection of 55 mg/kg streptozotocin via the penile vein after being anesthetized with pentobarbital (50 mg/kg IP); the remaining group received a single injection of the streptozotocin vehicle (citrate buffer) and served as the control group. Two weeks after the injection of streptozotocin, diabetes was confirmed by the presence of polydipsia, polyuria, and glycosuria. One group of diabetic rats was then injected daily with 5 IU subcutaneous insulin (Insulatard NPH HM, 40 IU/mL, Novo Nordisk A/S) in the afternoon for 3 weeks (SI group); the other group of diabetic rats and the control group were injected daily with subcutaneous saline (SS group and control group, respectively).

Fructose-Fed Rats

Four-week-old rats were randomly divided into 2 groups: the control group received a standard diet (FC group), and the fructose-fed group received a diet in which fructose composed 56.8% of the total carbohydrates (F group) for 8 weeks.

The day before the experiment, urine samples for each animal were collected for 24 hours. Urinary glucose was measured with a test strip (Keto-DiaburTest 5000, Roche), and samples were frozen at −80°C for later measurement of LTE4 excretion.

After intraperitoneal injection of sodium pentobarbital (50 mg/kg), the mean arterial blood pressure (MABP) was measured as previously described. Plasma glucose was measured with a test strip (Accu-Chek Easy, Boehringer-Mannheim, GB) for each rat. Plasma insulin level was measured as previously described by radioimmunoassay (Insulin CT, ORIS) for the F and FC groups. The thoracic aorta was then excised, transfused to a dish filled with Krebs bicarbonate buffer, cleared of periadventitial tissue, and cut into ring segments (3.0 mm in length). In certain rings, the endothelium was removed by gentle rubbing of the intimal surface with small forceps; in the remaining rings, care was taken not to touch the inner surface of the blood vessels.

Measurement of Isometric Tension in Rings of Aorta

Studies of isometric tension development were performed on aortic ring segments by the use of a method previously reported. The rings of aorta were initially stretched to a given preload of 1.5 g. After 60 minutes of equilibration, experiments were initiated by obtaining in each ring a reference contraction in response to KCl (90 mmol/L). The endothelial function was assessed by testing the relaxant effect of acetylcholine (1 μmol/L to 0.1 mmol/L) on aortic rings precontracted with phenylephrine (10 nmol/L to 0.1 μmol/L). Subsequently, the rings were allowed to equilibrate for another hour, with the Krebs solution being changed every 15 minutes. Cumulative concentration-response curves for Ang II (0.5-log increments, 10 ppmol/L to 0.3 μmol/L) were constructed after incubation of the preparations with either the specific 5-lipoxygenase inhibitor (AA861, 10 μmol/L for 30 minutes) or with the specific CysLT1 receptor antagonist (MK571, 1 μmol/L for 30 minutes). Rings from SS rats were also incubated with a CysLT1 receptor antagonist (BAY-u9773, 0.1 and 0.3 μmol/L for 30 minutes). In a separate series of experiments, the contractile effects of the CysLTs were also assessed in intact or endothelium-denuded aortic rings from the control, SS, and SI groups. Cumulative concentration-response curves were constructed for either LTC4, LTD4, or LTE4 (1-log increment, 10 nmol/L to 0.1 μmol/L) in the absence of AA861 (10 μmol/L) to avoid endogenous leukotriene production. In a further series of experiments, concentration-response curves for norepinephrine (1 nmol/L to 10 μmol/L) were elicited on intact aortic rings from SS rats for study of the selective effect of AA861 (10 μmol/L for 30 minutes) on Ang II–induced contraction.

Appropriate controls (incubation with solvents) were run under similar experimental conditions in rings obtained from the same aorta. Only 1 cumulative concentration-response curve was established for each agonist on any ring.

Measurement of CysLT Release in Rat Aortic Rings

The release of CysLTs in response to Ang II was measured as previously described. Briefly, intact aortic rings from all rat groups were incubated with either Ang II (0.3 μmol/L for 30 minutes), AA861 (10 μmol/L for 30 minutes) plus Ang II (0.3 μmol/L for 30 minutes), or solvents (control values). CysLT production was also measured on endothelium-denuded ring vessels from control, SS, and SI rats after challenge with Ang II (0.3 μmol/L for 30 minutes) or vehicle. A further series of experiments was performed to study the Ang II receptor subtype involved in Ang II–mediated CysLT release. Intact aortic rings from the control, SS, and SI groups were preincubated with the Ang II type 1 (AT1) receptor antagonist losartan (1 μmol/L) or with the Ang II type 2 (AT2) receptor antagonist (PD123319, 0.1 μmol/L) for 30 minutes before challenge with the submaximal concentration of Ang II (0.3 μmol/L) for another 30 minutes. Another series of experiments was performed to investigate the tyrosine kinase activity of insulin on Ang II–mediated CysLT release. Intact aortic rings from SS rats were preincubated with either insulin (Actrapid 70 μU/mL for 10 minutes), the tyrosine kinase activator insulin-like growth factor-1 (IGF-1, 1 ng/mL for 10 minutes), or vehicle (control value) and then challenged with Ang II (0.3 μmol/L) for 30 minutes. In a last series of experiments, the effect of the calcium ionophore (A23187, 10 μmol/L for 30 minutes) on CysLT production was also assessed in intact aortic rings from the control, SS, and SI groups. The Krebs solution was collected, and samples were frozen at −80°C. The rings were dried in an oven for measurement of dry weight. CysLTs were measured by enzyme immunoassay on unextracted samples by using reagents purchased from Cayman. The detection limit of the assay was 3.2 pg/mL, the EC50 (50% B/B0) was 36.6 pg/mL, and the intra-assay and interassay coefficients of variation were <10%.

Measurement of LTE4 Urinary Excretion

Measurement of LTE4 was performed as previously described on 5 mL urine. Extraction was performed by using C18 (EC) at 100 mg/10 mL (International Sorbent Technology, GB), and leukotrienes were finally eluted with methanol. The urine LTE4 levels were measured by enzyme immunoassay (Cayman). The detection limit of the assay was 36.1 pg/mL, the IC50 was 222.9 pg/mL, and the intra-assay and interassay coefficients of variation were <10%.

Statistical Analysis

Contractile responses were expressed as percentage of the contraction induced by 90 mmol/L KCl. The maximal effect (Emax) was the greatest response obtained with the agonist. The concentration of agonist producing 50% of the maximal effect (EC50) was determined from each curve by a logistic curve-fitting equation. The pD2 value is the negative logarithm of the EC50. The CysLT data were expressed as percentage over the control values. The data of the control values were given as picograms per milligram dry weight tissue. The urinary LTE4 data were expressed as picograms per 24 hours. Results are expressed as mean±SEM for the specified number of preparations tested. Statistical analysis were performed by...
ANOVA for repeated measures, followed by the Bonferroni-corrected $t$ test. Individual comparisons were made by the Student $t$ test for unpaired data. Correlation analysis was performed by a Spearman test. A value of $P<0.05$ was considered significant.

**Drugs**

Drugs used and their sources were as follows: KCl and ethanol were from Prolabo; Ang II, phenylephrine, acetylcholine, norepinephrine, AA861, IGF-1, EDTA, and the calcium ionophore A23187 were from Sigma; LTC$	ext{ }_{4}$, LTD$	ext{ }_{4}$, LTE$	ext{ }_{4}$, and MK571 were purchased from Cayman; BAY-u9773 was from Biomol; and insulin (Insulatard NPH HM at 40 UI/mL and Actrapid at 100 UI/mL) was from Novo Nordisk A/S. Losartan (Dup 753) and PD123319 were kindly provided by Merck (Canada) and Research Biochemicals International (United States), respectively. Drugs were kept at $-20^\circ$C and freshly dissolved in distilled water to the appropriate concentration expressed as final molar concentration in the organ bath.

**Results**

**Biological Data and MABP**

**Control, SS, and SI Groups**

Body weight, glycemia, urinary glucose, and 24-hour urine volume were significantly different between control and SS rats (Table 1). In SS rats, 3 weeks of daily injection of insulin rendered body weight, glycemia, and the urine volume comparable to those of control rats; however, the glycosuria still remained positive for 7 of 19 SI rats, with or without endothelium (data not shown). The MABP was not significantly different between each group (Table 1).

**FC and F Groups**

Body weight, glycemia, glycosuria, 24-hour urine volume, and MABP were comparable between the FC and F groups. In contrast, insulinemia was significantly higher in the F group (Table 1).

**Organ Chamber Experiments**

**Control, SS, and SI Groups**

The contraction elicited by 90 mmol/L KCl was significantly reduced in intact and endothelium-denuded aortic rings from SS rats compared with rings from control rats and SI rats. For intact aortic rings, $E_{\text{max}}$ values of 90 mmol/L KCl were $2839\pm158$ mg (control group, $n=18$), $1717\pm98$ mg (SS group, $n=30$), and $2358\pm143$ mg (SI group, $n=23$), with $P<0.0001$ for SS group versus control group and versus SI group. For endothelium-denuded rings, $E_{\text{max}}$ values of 90 mmol/L KCl were $1812\pm131$ mg (control group, $n=20$), $1305\pm67$ mg (SS group, $n=27$), and $1708\pm116$ mg (SI group, $n=32$), with $P<0.01$ for SS group versus control group and versus SI group.

Ang II induced concentration-dependent contractions of isolated aortic rings from control, SS, and SI rats. In terms of potency ($pD_{2}$), the response to Ang II was similar in rings with or without endothelium from control, SS, and SI rats (Table 2). In each group, the contractions elicited by Ang II were significantly increased in endothelium-denuded aortic rings ($P<0.003$, ANOVA). In contrast, the Ang II-evoked contractions were weaker in intact aortic rings from SI rats than from control rats (Table 2). The maximal contractions elicited by Ang II were similar in endothelium-denuded rings from control, SS, and SI rats (Table 2).

The 5-lipoxygenase inhibitor (AA861, 10 μmol/L) did not affect the resting tone of intact or endothelium-denuded isolated aortas from control, SS, and SI rats. However, AA861 significantly reduced the amplitude of the contraction elicited by Ang II in aortas from SS rats with or without endothelium (Figure, Table 2). In contrast, AA861 did not affect Ang II–induced contraction in aortic rings with or without endothelium from control and SI rats (Figure, Table 2).

The CysLT1 receptor antagonist (MK571, 1 μmol/L), which by itself had no effect on the basal tone, did not significantly modify the contraction elicited by Ang II in intact or endothelium-denuded aortas from control, SS, and SI rats (Table 2). In addition, the dual CysLT1/CysLT2 receptor antagonist (BAY-u9773, 0.1 μmol/L) was ineffective on the resting tone and on the contraction elicited by Ang II on aortas with or without endothelium from SS rats (Table 2). A higher concentration of BAY-u9773 (0.3 μmol/L) still did not affect either the basal tone or Ang II–induced contraction on aortic rings from SS rats (data not shown).

LTC$	ext{ }_{4}$, LTD$	ext{ }_{4}$, and LTE$	ext{ }_{4}$, elicited either weak or no contraction on intact and endothelium-denuded aortic rings from control and SI rats ($n=3$ for each leukotriene). In aortic rings from SS rats, LTC$	ext{ }_{4}$ and LTD$	ext{ }_{4}$ at a concentration of 0.1 μmol/L elicited weak but stable contractions. In rings with intact endothelium, the contractions elicited by LTC$	ext{ }_{4}$ and LTD$	ext{ }_{4}$ were $49.2\pm47.2$ mg (control, $n=2$) and $51.7\pm33.6$ mg (SI, $n=3$), respectively. In rings without endothelium, the contractions elicited by LTC$	ext{ }_{4}$ and LTD$	ext{ }_{4}$ were $41.9\pm5.2$ mg (control, $n=2$) and $22.9\pm9.3$ mg (SI, $n=6$), respectively. LTE$	ext{ }_{4}$ elicited either weak or no contraction in aortic rings from SS rats with or without endothelium (data not shown).

In terms of potency, AA861 (10 μmol/L) induced no significant change on norepinephrine-induced contraction: for pD$_{2}$, 7.5±0.1 (control, $n=6$) versus 7.4±0.2 (AA861, $n=6$). However, AA861 (10 μmol/L) significantly reduced by $\approx18.1\%$ the maximal contraction induced by norepineph-

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>MABP, mm Hg</th>
<th>Glycemia, mg/dL</th>
<th>Insulinemia, μU/mL</th>
<th>Urinary Glucose, g/dL</th>
<th>24-h Urine Volume, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ($n=17$)</td>
<td>399.4±8.7</td>
<td>131.1±3.7</td>
<td>141.2±8.1</td>
<td>ND</td>
<td>0±0</td>
<td>18.6±3.4</td>
</tr>
<tr>
<td>SS ($n=31$)</td>
<td>283.4±6.3$^\dagger$</td>
<td>121.2±3.8</td>
<td>440.0±19.8$^\dagger$</td>
<td>ND</td>
<td>4.9±0.1$^\dagger$</td>
<td>94.2±8.4$^\dagger$</td>
</tr>
<tr>
<td>SI ($n=19$)</td>
<td>395.5±6.3</td>
<td>130.8±3.3</td>
<td>182.2±34.2</td>
<td>ND</td>
<td>1.3±0.5</td>
<td>15.2±4.1</td>
</tr>
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<td>FC ($n=10$)</td>
<td>364.0±12.9</td>
<td>134.4±6.1</td>
<td>126.0±6.7</td>
<td>43.2±7.4</td>
<td>0.2±0.1</td>
<td>8.7±1.7</td>
</tr>
<tr>
<td>F ($n=10$)</td>
<td>365.4±10.2</td>
<td>135.0±5.7</td>
<td>133.6±6.5</td>
<td>77.1±12$\dagger$</td>
<td>0.1±0.1</td>
<td>16.2±3.0</td>
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</table>

ND indicates not determined. Values are mean±SEM for the specified number of animals tested ($n$). *$P<0.05$ vs control; †$P<0.05$ vs SI; and ‡$P<0.05$ vs FC.
TABLE 2. pD2 and Emax of Ang II–Induced Contractions in Aortas From Control, SS, and SI Rats With or Without Endothelium in the Absence or the Presence of AA861 (10 μmol/L), MK571 (1 μmol/L), and BAY-u9773 (0.1 μmol/L)

<table>
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<th></th>
<th>Control</th>
<th>SS</th>
<th>SI</th>
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<tr>
<td>Ang II</td>
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<tr>
<td>pD2</td>
<td>7.8±0.1</td>
<td>7.8±0.1</td>
<td>7.9±0.2</td>
</tr>
<tr>
<td>Emax, %</td>
<td>20.9±5.8</td>
<td>24.0±5.5</td>
<td>12.7±3.0</td>
</tr>
<tr>
<td>Emax, mg</td>
<td>558.0±165.0</td>
<td>389.2±91.3</td>
<td>238.7±43.7*</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>AA861</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pD2</td>
<td>7.9±0.1</td>
<td>8.2±0.1</td>
<td>8.1±0.3</td>
</tr>
<tr>
<td>Emax, %</td>
<td>22.7±1.5</td>
<td>13.2±4.1†</td>
<td>11.6±2.6</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>MK571</td>
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<tr>
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<td>11.1±3.7</td>
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<tr>
<td>n</td>
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<td>7</td>
</tr>
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<tr>
<td>pD2</td>
<td>ND</td>
<td>7.6±0.2</td>
<td>ND</td>
</tr>
<tr>
<td>Emax, %</td>
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<td>20.7±2.1</td>
<td>ND</td>
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<td>Ang II</td>
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<td>7.9±0.1</td>
<td>8.0±0.2</td>
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<td>Emax, mg</td>
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<td>408.3±85.2</td>
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<td>8</td>
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<td>13</td>
</tr>
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<td>AA861</td>
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<tr>
<td>pD2</td>
<td>7.8±0.1</td>
<td>8.0±0.1</td>
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<tr>
<td>Emax, %</td>
<td>34.5±2.7</td>
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<td>26.6±6.4</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
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</tr>
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<td>Emax, %</td>
<td>29.4±4.8</td>
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<td>pD2</td>
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<td>7.9±0.1</td>
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<td>37.7±9.0</td>
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*pD2 indicates potency; Emax, efficacy (expressed as percentage of the contraction elicited by 90 mmol/L KCl or as milligrams of isometric force); and n, number of aortic rings. Values are mean±SEM of experiments performed on n = 6. AA861 (10 μmol/L) and MK571 (1 μmol/L) failed to modify the contraction elicited by Ang II in intact aortas from the FC and F groups, respectively. Similarly, MK571 (1 μmol/L) failed to modify the contraction elicited by Ang II in intact aortas from the FC and F groups, respectively. In the presence of MK571, the pD2 values of Ang II were 7.5±0.2 (n=5) and 7.9±0.1 (n=8) in aortas from the FC and F groups, respectively, and the Emax values were 18.5±1.4% (n=5) and 20.5±3.1% (n=8) in aortas from the FC and F groups, respectively. Similarly, MK571 (1 μmol/L) failed to modify the contraction elicited by Ang II in intact aortas from the FC and F groups, respectively. In the presence of MK571, the pD2 values of Ang II were 7.7±0.2 (n=6) and 8.0±0.1 (n=6) in aortas from the FC and F groups, respectively, and the Emax values were 14.2±1.4% (n=6) and 21.1±5.3% (n=6) for the FC and F groups, respectively.

**FC and F Groups**
The contractions elicited by 90 mmol/L KCl were similar in intact aortic rings from the FC group (2409±193 mg, n=11) and the F group (2368±148 mg, n=11).

In terms of potency and efficacy, Ang II elicited similar contraction on aortic rings from the FC and F groups: for pD2, 7.7±0.1 (FC group, n=11) versus 7.9±0.1 (F group, n=11); for Emax, 23.7±3.6% (FC group, n=11) versus 24.2±3.8% (F group, n=11).

AA861 (10 μmol/L) had no significant effect on the resting tone of intact aortas from the FC and F groups and induced no modification on Ang II contraction. In the presence of AA861, the pD2 values of Ang II were 7.5±0.2 (n=5) and 7.9±0.1 (n=8) in aortas from the FC and F groups, respectively, and the Emax values were 18.5±1.4% (n=5) and 20.5±3.1% (n=8) in aortas from the FC and F groups, respectively. Similarly, MK571 (1 μmol/L) failed to modify the contraction elicited by Ang II in intact aortas from the FC and F groups, respectively. In the presence of MK571, the pD2 values of Ang II were 7.7±0.2 (n=6) and 8.0±0.1 (n=6) in aortas from the FC and F groups, respectively, and the Emax values were 14.2±1.4% (n=6) and 21.1±5.3% (n=6) for the FC and F groups, respectively.

**Release of CysLTs in Rings of Rat Aorta**

**Control, SS, and SI Groups**
Ang II induced a significant increase over the control values of the CysLT production in aortic rings from SS rats, with and
TABLE 3. Release of CysLTs From Control, SS, and SI Aortic Rings With or Without Endothelium in the Absence (Control Values) or Presence of Ang II (0.3 μmol/L), AA861 (10 μmol/L)+Ang II (0.3 μmol/L), Losartan (1 μmol/L)+Ang II (0.3 μM), or PD123319 (0.1 μmol/L)+Ang II (0.3 μmol/L)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SS</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang II</td>
<td>145±6</td>
<td>636±146*</td>
<td>113±22</td>
</tr>
<tr>
<td>Ang II+Ang II</td>
<td>123±4</td>
<td>190±74†</td>
<td>50±13</td>
</tr>
<tr>
<td>Losartan+Ang II</td>
<td>85±14</td>
<td>181±51†</td>
<td>50±46</td>
</tr>
<tr>
<td>PD123319+Ang II</td>
<td>124±66</td>
<td>563±230*</td>
<td>89±47</td>
</tr>
<tr>
<td>% of control</td>
<td>101%</td>
<td>19%</td>
<td>51%</td>
</tr>
</tbody>
</table>

Values are expressed as percentage over respective control values and are presented as mean±SEM. % indicates number of aortic rings. Values are expressed as percentage over respective control values and are presented as mean±SEM. In rings without endothelium, the control values were as follows: 28.5±5.77 (n=6), 24.6±4.4 (n=9), and 40.4±5.9 (n=6) for control, SS, and SI rats, respectively. In rings without endothelium, the control values were as follows (pg/mg dry wt tissue): 28.9±8.6 (n=4), 35.4±8.9 (n=8), and 31.5±11.3 (n=5) for control, SS, and SI rats, respectively. *P<0.05 vs respective control values; †P<0.05 vs Ang II.

without endothelium (Table 3). In intact aortic rings, this Ang II–mediated increase in CysLT production was prevented by the 5-lipoxygenase inhibitor AA861 and by the AT1 receptor losartan but not by the AT2 receptor antagonist PD123319 (Table 3). In addition, the tyrosine kinase activators insulin and IGF-1 also inhibited the increase in CysLT production mediated by Ang II. The CysLT levels, expressed as percentages over the control value (27.2±10.3 pg/mg dry wt tissue, n=6), were as follows: 89±19% (IGF-1 plus Ang II, n=7), P<0.05 versus Ang II (636±146%), and 89±19% (insulin plus Ang II, n=6), P<0.05 versus Ang II (636±146%).

In contrast, in intact or endothelium-denuded aortic rings from control and SI rats, Ang II failed to modify CysLT production, and neither AA861 nor losartan nor PD123319 had an influence on CysLT release (Table 3).

The calcium ionophore A23187 induced a significant (P<0.05) increase over the respective control values in CysLT production in intact rat aortas from the control group (520±211%, n=4), SS group (429±49%, n=4), and SI group (276±101%, n=8).

**CF and F Groups**

Ang II did not significantly modify the CysLT release either in intact aorta from the FC or F groups. The CysLT production over control values was 119±19% for the FC group (n=6) and 162±41% for the F group (n=6). The control values were (28.6±1.8 pg/mg dry wt [FC group, n=6] and 38.3±11.7 pg/mg dry wt [F group, n=6]).

**Measurement of LTE4 Urinary Levels for Control, SS, and SI Groups**

LTE4 urinary levels were significantly increased in the SS group compared with the control group (1.5±0.5 ng/24 h [control group, n=6] versus 13.7±2.9 ng/24 h [SS group, n=10], P<0.0004) and were positively correlated with glycemia (r=0.46, P<0.01). In addition, the LTE4 urinary level for SI rats was similar to that for control rats (1.5±0.4 ng/24 h [SI group, n=9]).

**Discussion**

The results of the present study provide evidence for the activation of the 5-lipoxygenase pathway in SS rats and suggest the involvement of 5-lipoxygenase–derived products in the in vitro constriction induced by Ang II in isolated aortas from SS rats.

**Biological Data**

The streptozotocin-induced diabetic rat is an experimental model of insulin-dependent diabetes, which displays many of the features seen in human subjects with uncontrolled diabetes mellitus, including hyperglycemia, polydipsia, polyuria and weight loss. The diabetogenic effect of streptozotocin is due to its selective toxicity for pancreatic β cells, which leads to the loss of insulin secretion. Chronic treatment with insulin during 3 weeks rendered the body weight, glycemia, and 24-hour urine volume of SI rats comparable to those of control rats.

In addition, rats fed with high dosages of fructose develop insulin resistance and provide a useful experimental model of insulin resistance.

**Contractile Response to 90 mmol/L KCl and Ang II in Aortas From Control, SS, and SI Rats**

The cardiovascular system of diabetic rats is characterized by functional alterations compared with that of control rats. In the present study, the contraction elicited by KCl was significantly depressed in aortas from SS rats. However, the contractions elicited by Ang II in SI rats were not significantly decreased in aortic rings from SS rats as previously reported. Three-week insulin treatment reduced the amplitude of the contraction elicited by Ang II in intact aortic rings from SI rats compared with control rats. These data are consistent with the inhibitory effect of insulin on the contractile response to Ang II reported in rabbit femoral arteries and veins. Ang II–mediated contraction is negatively modulated by endothelium-derived NO, and insulin has been shown to increase endothelial NO synthase (eNOS) expression and activity in bovine aortic endothelial cells and, thereby, to increase NO production.

Therefore, in our experimental conditions, the permissive insulin-stimulated release of NO may help to explain, at least in part, the reduced contractile response to Ang II in intact aortas from insulin-treated rats. However, this inhibitory effect of insulin on Ang II–mediated contraction was not found in intact aortas from the F group. Pertaining to this finding, insulin failed to increase eNOS mRNA expression in the vascular stroma of another insulin-resistant model (Zucker fatty rats). Collectively, these data suggest that insulin resistance may be associated with a blunted effect of insulin on eNOS expression. The resulting loss of NO vasodilator effects may help to explain, at least in part, the unchanged contractile response to Ang II in aortas from the F group compared with the FC group.

**Effect of a 5-Lipoxygenase Inhibitor on Ang II–Induced Contraction**

The main finding of the present study is the inhibitory effect of AA861, a specific 5-lipoxygenase inhibitor, on Ang II–mediated vasoconstriction in isolated aortas from SS rats.
II–induced contraction in aortas with or without endothelium from the SS group but not from the control, SI, FC, and F groups. These results are in line with the previous demonstration of the involvement of the 5-lipoxygenase pathway in Ang II–mediated contraction in aortas from spontaneously hypertensive rats but not from control Wistar-Kyoto rats and in human internal mammary arteries taken from patients undergoing cardiovascular bypass surgery. The role of 5-lipoxygenase–derived products has also been reported in Ang II–mediated aldosterone production in rat glomerulosa cells. However, only a part of Ang II–induced contraction was inhibited by AA861, suggesting that metabolites other than 5-lipoxygenase–derived products could be involved in Ang II–evoked contraction. In this regard, TP receptor agonists have also been reported to contribute to the contraction elicited by Ang II.

In addition, the finding that norepinephrine-induced contraction was reduced by AA861 suggested that 5-lipoxygenase–derived products could also contribute to the constrictor effects of norepinephrine. These data are in agreement with a previous work reporting a release of leukotrienes in response to norepinephrine in humans. However, in aortas from SS rats, the inhibitory effect of AA861 was weaker on norepinephrine-induced contraction than on Ang II–induced contraction.

CysLT Production in Aortas From Control, SS, and SI Rats

The production of CysLTs by aortic rings was determined with specific enzyme immunoassay. In rings with endothelium from SS rats, Ang II induced a significant increase in CysLT release. Moreover, the observation that the Ang II–mediated CysLT release was inhibited by the 5-lipoxygenase inhibitor AA861 confirmed the finding that the materials released by the aortic rings in response to Ang II were 5-lipoxygenase–derived products. Diabetes mellitus has been associated with alteration of eicosanoid release in response to Ang II. Vascular smooth muscle cells cultured under high glucose conditions have an increased expression of 12-lipoxynagenase protein and an increased production of 12-HETE in response to Ang II. The present study has demonstrated that in addition to the release of 12-HETE, Ang II also stimulates the release of CysLTs. Leukotrienes can be formed from arachidonic acid by perivascular mast cells and monocytes/macrophages but can also be synthesized by platelets and endothelial and vascular smooth muscle cells from neutrophil-derived LTA4. In the present study, immunohistochemical staining suggests the absence of monocytes/macrophages in the vessel wall (data not shown). In addition, the Ang II–mediated CysLTs that were released were not modified by endothelium removal. Collectively, these data suggest that in our experimental conditions, CysLT biosynthesis did not take place at the level of the monocytes/macrophages or the endothelial cells. It has been reported that cultured smooth muscle cells from rabbit, porcine, or human vascular preparations were able to produce 5-lipoxygenase–derived products. However, the synthesis of CysLTs has not been definitively demonstrated at the level of the smooth muscle cells, and the cellular localization of CysLT synthesis requires further studies. In contrast with SS rats, no significant modification of the CysLT production was observed after Ang II stimulation in aortas from control and SI rats, although aortic ring stimulation with the calcium ionophore confirmed the capacity of the vascular tissue to produce CysLTs.

Therefore, the contribution of the AT1 and AT2 receptors to the Ang II–induced release of CysLTs in SS rats has been studied by using the selective AT1 and AT2 receptor antagonists, losartan and PD123319, respectively. Pretreatment with losartan but not with PD123319 prevented the increased production of CysLTs in response to Ang II, indicating that Ang II was acting through the activation of AT1 receptors. These data are consistent with previous works reporting an increased lipoxygenase activity through the activation of AT1 receptors located on vascular smooth muscle cells.

Chronic treatment with insulin inhibited the Ang II–mediated CysLT production from aortas. In this regard, it has been reported in cultured vascular smooth muscle cells that activation of tyrosine kinase signaling pathways with insulin or IGF-I may function as a negative modulator of Ang II–induced intracellular free calcium concentration and contractile responses. In addition, intracellular calcium concentration modulates 5-lipoxygenase activation. Therefore, the inhibitory effect of insulin on Ang II–mediated CysLTs may be explained by its inhibitory effect on Ang II–induced calcium release. This hypothesis has been confirmed by the finding that short-term incubation with insulin or another tyrosine kinase activator, IGF-1, prevented the in vitro CysLT production elicited by Ang II in aortic rings from SS rats. Moreover, the inhibitory effect of insulin on Ang II–mediated CysLT release is reinforced by the observation that Ang II did not modify in vitro CysLT production in aortas from the F group. In line with these findings, short-term exposure of human adrenal glomerulosa cells with insulin has been shown to reduce Ang II–mediated 12-HETE release.

LTE4 Excretion in Control, SS, and SI Rats

Abnormalities in the arachidonic acid cascade to either the cyclooxygenase or lipoxygenase pathways have been observed in diabetic animal models and humans. In an attempt to determine whether diabetes mellitus was associated with activation of the 5-lipoxygenase pathway, we studied the urinary excretion of LTE4 in control, SS, and SI rats. LTE4 excretion was significantly increased in SS rats and positively correlated with glycemia. In agreement with this finding, an increased LTB4 plasma level, positively correlated with glycated hemoglobin, has been reported in type I and II diabetic patients. Collectively, these data suggest an activation of the 5-lipoxygenase pathway in diabetes mellitus. In contrast, the urinary excretion of LTE4 in SI rats was similar to that in control rats, confirming the inhibitory influence of insulin on CysLT production.

Effect of CysLT1 and a CysLT1/2 Antagonists on Ang II–Induced Contraction

The contribution of the CysLT1 receptors to Ang II–induced contraction was then studied by using the selective CysLT antagonist MK571 and the CysLT1/2 antagonist BAY-u9773. Ang II–induced contraction on aortic rings from the control, SS, SI, FC, and F groups was not modified with prior incubation with MK571. Furthermore, BAY-u9773 did not alter the Ang II–induced contraction of aortas from the SS group. These observations suggest that in isolated aortas from
the SS group, CysLTs may contribute to the contraction elicited by Ang II through stimulation of a CysLT receptor different from the well-characterized CysLT1 or CysLT2 receptors. The existence of such an undefined CysLT receptor, termed the non-CysLT1-CysLT2 receptor, has recently been suggested by Bäck and colleagues, who reported that the contractile response to the CysLTs remained resistant to CysLT1 and CysLT2 receptor antagonists in human and porcine pulmonary arteries. In the present study, LTC4 and LTD4 elicited small contractions, which did not allow the definitive characterization of the CysLT receptor involved in the contractile effects of Ang II. However, these CysLT-induced contractions were in the range of 10% to 13% of the Ang II–induced contractions.

Conclusions
In conclusion, the present study strongly supports the activation of the 5-lipoxygenase pathway in SS rats and the involvement of 5-lipoxygenase–derived products and, particularly, the CysLTs in Ang II–induced contraction in aortas of SS rats. Results obtained with SI rats suggest an inhibitory influence of insulin on vascular CysLT production in response to Ang II but also on the urinary excretion of LTE4.

The finding that the 5-lipoxygenase pathway is involved in Ang II–mediated contraction is of particular interest in the area of diabetic vascular complications, because an increased activation of the 5-lipoxygenase pathway has been reported in patients with coronary artery disease and cardiac ischemia and because CysLTs have been shown to contract human atherosclerotic coronary arteries. With regard to the involvement of proinflammatory mediators in diabetes, these data open the field of the potential clinical interest of 5-lipoxygenase pathway inhibition for the prevention of cardiovascular complications associated with diabetes mellitus.

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References
Cysteinyl Leukotrienes Modulate Angiotensin II Constrictor Effects on Aortas From Streptozotocin-Induced Diabetic Rats
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