Role of ET-1 receptor binding and $[\text{Ca}^{2+}]_i$ in contraction of coronary arteries from DOCA-salt hypertensive rats

ARARAT D. GIULUMIAN,1 MARIELA M. MOLERO,1 VIKRAM B. REDDY,2 JENNIFER S. POLLOCK,1,2 DAVID M. POLLOCK,1,3 AND LESLIE C. FUCHS1,2

1Vascular Biology Center, 2Department of Pharmacology and Toxicology, and 3Department of Surgery, Medical College of Georgia, Augusta, Georgia 30912-2500

Received 18 July 2001; accepted in final form 26 December 2001

Giulumian, Ararat D., Mariela M. Molero, Vikram B. Reddy, Jennifer S. Pollock, David M. Pollock, and Leslie C. Fuchs. Role of ET-1 receptor binding and $[\text{Ca}^{2+}]_i$, in contraction of coronary arteries from DOCA-salt hypertensive rats. Am J Physiol Heart Circ Physiol 282: H1944–H1949, 2002; 10.1152/ajpheart.00627.2001.—Hypertension is associated with an increase in coronary artery disease, but little is known about the regulation of coronary vascular tone by endothelin-1 (ET-1) in hypertension. The present study evaluated the mechanisms mediating altered contraction to ET-1 in coronary small arteries from deoxycorticosterone acetate (DOCA)-salt hypertensive rats. DOCA-salt rats exhibited an increase in systolic blood pressure and testosterone acetate (DOCA)-salt hypertensive rats. DOCA-contraction to ET-1 in coronary small arteries from deoxycorticosterone acetate (DOCA)-salt hypertensive rats. DOCA-salt rats exhibited an increase in systolic blood pressure and plasma ET-1 levels compared with placebo rats. Contraction to ET-1 ($1 \times 10^{-11}$ to $3 \times 10^{-8}$ M), measured in isolated coronary small arteries maintained at a constant intraluminal pressure of 40 mmHg, was largely reduced in vessels from DOCA-salt rats compared with placebo rats. To determine the role of endothelin receptor binding in the impaired contraction to ET-1, $^{125}$I-labeled ET-1 receptor binding was measured in membranes isolated from coronary small arteries. Maximum binding (fmol/mg protein) and binding affinity were similar in coronary membranes from DOCA-salt rats compared with placebo rats. Changes in intracellular $\text{Ca}^{2+}$ concentration ($[\text{Ca}^{2+}]_i$) were measured in freshly dissociated coronary small arteries smooth muscle cells loaded with fura 2. ET-1 ($10^{-9}$ M) produced a 30% increase in $[\text{Ca}^{2+}]_i$, in smooth muscle cells from placebo rats, but had no effect on cells from DOCA-salt rats (2% increase). In summary, the ET-1-induced coronary artery contraction and increase in $[\text{Ca}^{2+}]_i$, are impaired in DOCA-salt hypertensive rats, whereas endothelin receptor binding is not altered. These results suggest endothelin receptor uncoupling from signaling mechanisms and indicate that impaired $[\text{Ca}^{2+}]_i$, signaling contributes to the decrease in ET-1-induced contraction of coronary small arteries in DOCA-salt hypertensive rats.

**ENDOTHELIN-1 (ET-1)** is a potent vasoconstrictor peptide thought to contribute to several cardiovascular diseases, including hypertension and ischemia-reperfusion injury (2, 28). Vascular contraction induced by ET-1 is most commonly mediated by stimulation of smooth muscle cell ET$_A$ receptors but may also occur via activation of smooth muscle cell ET$_B$ receptors (26, 32). ET-1 also stimulates ET$_B$ receptors located on vascular endothelium to produce vasodilation through release of nitric oxide and prostacyclin (5). Calcium entry into smooth muscle cells from the extracellular space through voltage-gated $\text{Ca}^{2+}$ channels and intracellular $\text{Ca}^{2+}$ release from the sarcoplasmic reticulum contribute to the initiation and maintenance of smooth muscle contraction (14). ET-1 has been shown to increase extracellular influx of $\text{Ca}^{2+}$ and to release intracellular $\text{Ca}^{2+}$ from the sarcoplasmic reticulum through the inositol 1,4,5-trisphosphate signaling pathway (16, 26, 29).

An increase in plasma ET-1 levels has been reported in several pathophysiological conditions including hypertension (28). Vascular endothelial cell ET-1 mRNA expression is elevated in the deoxycorticosterone acetate (DOCA)-salt rat model of hypertension, whereas contraction to ET-1 is decreased in many vascular beds in this model (6, 9, 10, 12, 20, 22, 24). Treatment of DOCA-salt hypertensive rats with either a combined ET$_A$-ET$_B$ receptor antagonist or a selective ET$_A$ receptor antagonist impairs the development of hypertension and reduces total peripheral resistance, vascular hypertrophy, and remodeling, suggesting an important role for ET-1 in this model of hypertension (1, 8, 20). Inhibition of ET$_A$-ET$_B$ receptors was also shown to decrease myocardial hypertrophy and fibrosis in DOCA-salt hypertensive rats (15). Additionally, in the left ventricular myocardium of DOCA-salt hypertensive rats, subendocardial arteriolar growth and capillary rarefaction were observed, and both were partially corrected by ET$_A$ receptor antagonism (19). This suggests a role for ET-1 in contributing to changes in the coronary microvasculature in this model of hypertension.

In a previous study, we measured contraction to ET-1 in DOCA-salt rats and found a dramatic reduction in contraction to ET-1 in coronary small arteries (200–300 $\mu$m intraluminal diameter (ID)) (9). The mechanisms mediating the changes in coronary artery contraction to ET-1 are unknown. Therefore, the present study was designed to determine the role of...
endothelin receptor binding and ET-1-induced changes in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) in the impaired contraction of coronary small arteries from DOCA-salt hypertensive rats.

METHODS

General procedures. Male Sprague-Dawley rats (200 g) (Harlan Laboratories; Indianapolis, IN) were uninephrectomized under methohexitol sodium (Brevital) anesthesia. During this procedure, DOCA-salt-treated rats were implanted with subcutaneous DOCA pellets (200 mg/rat) and given saline (0.9%) to drink ad libitum. Placebo rats were implanted with placebo pellets and given tap water to drink. After ~3 wk, systolic blood pressure was measured via tail cuff. Rats were then anesthetized with pentobarbital sodium (60 mg/kg ip), and arterial blood samples were obtained from the abdominal aorta for determination of plasma ET-1 concentrations by luminescent ELISA (R&D Systems; Minneapolis, MN). Heparin (100 units) was then administered via the left ventricle 3 min before the removal of the heart. Coronary small arteries were isolated for measurement of vascular reactivity, receptor binding, or calcium imaging as described below.

Vascular reactivity. The heart was placed in chilled, oxygenated (20% O\(_2\)-5% CO\(_2\)-balance N\(_2\)) Krebs-Ringer bicarbonate solution (composition of (in mM) 118.3 NaCl, 4.7 KCl, 2.5 CaCl\(_2\), 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 25 NaHCO\(_3\), and 11.1 dextrose). Coronary small arteries (50–300 μm, intraluminal diameter) were isolated and maintained at a constant intraluminal pressure of 40 mmHg during continuous measurement of ID with a video dimension analyzer (Living Systems Instrumentation) as previously described (9). This range of vessel ID was selected to correlate with the intraluminal diameter of arteries used for receptor binding assays and for smooth muscle cell isolation. Concentration-response curves to ET-1 (1 × 10\(^{-11}\) to 3 × 10\(^{-8}\) M) were performed in the absence or presence of the selective ET\(_A\) receptor antagonist A-127722 (30 nM) (34). A-127722 was added to the vessel bath 30 min before the concentration-response curve to ET-1 was performed. ID measurements obtained from coronary small arteries were expressed in micrometers. Vasoconstrictor responses in isolated vessels were expressed as percentage contraction. Only one experiment was performed per vessel, and each experiment was performed only once per rat.

Endothelin receptor binding. Binding characteristics of \(^{125}\)I-labeled ET-1 (\(^{125}\)I-ET-1), which represents the total number of endothelin receptors, were determined in coronary small artery membrane preparations in a manner similar to that described previously for the rat renal medulla (25). The heart was removed and placed in chilled, oxygenated (20% O\(_2\)-5% CO\(_2\)-balance N\(_2\)) modified Krebs buffer. Coronary small arteries (50–300 μm ID) were dissected and homogenized in ice-cold homogenizing solution containing 250 mM sucrose, 50 mM Tris-HCl, pH 7.4, 5 mM EDTA, and 25 μM phenylmethylsulfonyl fluoride (PMSF) in a glass/glass homogenizer. The homogenate was centrifuged at 1,000 \(\times\) g for 30 min at 4°C. The resulting supernatant was centrifuged at 30,000 \(\times\) g for 45 min at 4°C. This supernatant was removed, and the pellet was resuspended in one-half the initial amount of homogenization buffer. Protein concentration was determined using the Bradford method (Bio-Rad; Hercules, CA).

A known quantity of membrane preparation was added to each well of a 96-well microtiter plate (Optiplate, Packard Instruments; Meridan, CT). Wheat germ agglutinin polivinyllotene beads (scintillation proximity beads, Amersham Life Sciences; Arlington Heights, IL) were suspended in binding buffer (40 mg/ml), and 1 mg was added to each well. Binding buffer (pH 7.4) was composed of (in mM) 20 Tris-HCl, 100 NaCl, and 10 MgCl\(_2\) and contained 0.1 mM PMSF, 5 μg/ml pepstatin A, 0.025% bacitracin, 3 mM EDTA, and 0.2% bovine serum albumin. The plate was covered and shaken gently for 2.5 h at room temperature. After this precoupling process, 25 μl of binding buffer were added to those wells required for total binding, whereas ET-1 was added to the other well (final concentration of 1 μM) for the nonspecific binding. \(^{125}\)I-ET-1 was added to each well for determination of specific binding. The plate was sealed and shaken gently for 18 h at room temperature. The plate was counted on a Packard TopCount scintillation counter.

The coronary artery membrane preparation that was bound to wheat germ agglutinin beads scintillates only when radioactive ligand is bound. With this scintillation proximity assay, separation of bound ligand from free is not required (11, 31). All points were performed in duplicate, and all dilutions of peptides were performed in siliconized tubes. Before a saturation binding curve was established, the amount of protein required was estimated by performing total and nonspecific binding for 0.1 nM \(^{125}\)I-ET-1 at each of the protein concentrations. To obtain enough protein for the binding curve, coronary arteries from five rats were pooled. Binding data were analyzed by nonlinear regression of the binding isotherm using Prism (GraphPad Software; San Diego, CA).

Changes in [Ca\(^{2+}\)]\(_i\) by measurement of fura 2. ET-1-induced changes in fura 2 fluorescence were measured in single smooth muscle cells freshly dispersed from coronary small arteries (50–300 μm ID). Enzymatic dispersion of smooth muscle cells was performed by placing coronary small arteries in 5 ml of dissociation medium (in mM: 110 NaCl, 5 KCl, 2 MgCl\(_2\), 0.16 CaCl\(_2\), 10 HEPES, 10 NaHCO\(_3\), 0.5 KH\(_2\)PO\(_4\), 0.5 NaH\(_2\)PO\(_4\), 0.49 EDTA, 10 taurine, and 10 glucose; pH, 6.9) containing albumin (4 mg/ml) with papain (1.52 mg/ml) and with dithiothreitol (0.54 mg/ml) added. Smooth muscle cells were dispersed for 30 min in a shaking water bath at 37°C followed by mild trituration. The solution was centrifuged at 1,000 g for 12 min, and the pellet was resuspended in fresh dissociation medium. Cells were loaded with 10 μM fura 2-AM and Pluronic F-127 (0.1%) (Molecular Probes) for 20 min at room temperature and washed three times with normal Ringer solution (in mM: 140 NaCl, 5 KCl, 1 MgCl\(_2\), 2 CaCl\(_2\), and 10 HEPES; pH 7.4). Cells were resuspended in normal Ringer solution and placed on a gelatin-coated coverslip in a chamber that was mounted on the stage of a microscope (Olympus IX70). Light from an ultraviolet light lamp passed to the cells through a rotating wheel containing 340- and 380-nm interference filters. The fluorescence emission at 510 nm was recovered using a digital camera and subtraction of background fluorescence and calculation of the ratio of fura 2 fluorescence (a relative measure of average myoplasmic free Ca\(^{2+}\) concentration [Ca\(^{2+}\)]\(_i\)) were performed with UltraView software (Life Science Resources; Cambridge, MA).

Data analysis. All data are reported as means ± SE. Statistical differences were determined by analysis of variance for repeated measures followed by Student’s modified t-test with Bonferroni correction for multiple comparisons.

Chemicals. ET-1, KCl, and ACh were obtained from Sigma Chemicals (St. Louis, MO). Wheat germ agglutinin Scintillation Proximity Assay beads were obtained from Amersham Life Sciences. \(^{125}\)I-ET-1 (2,200 Ci/mmole) was purchased from New England Nuclear (Boston, MA). A-127722 was supplied out of the courtesy of Dr. Jerry Wessale of Abbott Laboratories (North Chicago, IL).
RESULTS

Systolic blood pressure and plasma ET-1 levels measured in DOCA-salt and placebo rats are shown in Table 1. Systolic blood pressure and plasma ET-1 levels were significantly elevated in DOCA-salt rats compared with placebo rats.

Vascular reactivity. Figure 1A shows contraction to ET-1 (1 × 10⁻¹¹ to 3 × 10⁻⁸ M) in coronary small arteries with an average size of 104 ± 12 μm (range of 83–141 μm ID), whereas Fig. 1B shows contraction to ET-1 (1 × 10⁻¹¹ to 3 × 10⁻⁸ M) in coronary small arteries with an average size of 218 ± 18 μm (range of 162–285 μm ID). The maximum contraction to ET-1 was enhanced in placebo vessels ranging from 83–141 μm ID (82 ± 1%, Fig. 1A) compared with those of 162–285 μm ID (56 ± 6%, Fig. 1B). Additionally, the EC₅₀ (1 × 10⁻¹⁰ M) was significantly lower in vessels ranging from 83–141 μm compared with those ranging from 162–285 μm from placebo rats (1.8 ± 0.4 vs. 10.6 ± 1.3), indicating an enhanced sensitivity to ET-1 in smaller vessels. Contraction to ET-1 was dramatically reduced in vessels from DOCA-salt rats compared with vessels of similar size from placebo rats. Contraction to ET-1 was largely inhibited by the selective ETA receptor antagonist A-127722 in all groups (Fig. 1). Because functional changes were similar in the range of vessel size studied, a similar range of vessel ID (50–300 μm) was used for the studies on endothelin receptor binding and changes in [Ca²⁺]ᵢ described below.

Endothelin receptor binding. Receptor binding studies were conducted to determine whether the decreased contraction to ET-1 in coronary small arteries from DOCA-salt rats was due to reduced endothelin receptor binding. Initial binding experiments were conducted to characterize the method using coronary small arteries obtained from normal Sprague-Dawley rats. Binding for [¹²⁵I]ET-1 was measured over a range of membrane protein concentrations (0.5, 1, 2, and 5 μg/well), and maximum binding (B_max) was observed at 1 μg/well (Fig. 2A). Therefore, this concentration was used in subsequent experiments. Optimal equilibration time was found to be 18 h. A saturation binding curve and Scatchard plot were obtained for [¹²⁵I]ET-1 using membrane preparations from normal Sprague-Dawley rats (Fig. 2B).

B_max and dissociation constant (K_d) of [¹²⁵I]ET-1 in membrane preparations from coronary small arteries of placebo and DOCA-salt rats are shown in Table 2.

Table 1. Blood pressure and plasma ET-1 in placebo and DOCA-salt rats

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>DOCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>141 ± 6 (n = 10)</td>
<td>212 ± 10⁵* (n = 11)</td>
</tr>
<tr>
<td>Plasma ET-1, pg/ml</td>
<td>0.56 ± 0.08 (n = 7)</td>
<td>1.08 ± 0.14* (n = 8)</td>
</tr>
</tbody>
</table>

Values represent means ± SE; n, no. of rats. ET-1, endothelin-1; DOCA, deoxycorticosterone acetate. *P < 0.05 vs. placebo.

Fig. 1. Concentration-response curve to endothelin-1 (ET-1) in the presence or absence of the selective ETA receptor antagonist A-127722 (30 nM) in coronary small arteries from deoxycorticosterone acetate (DOCA)-salt hypertensive and placebo rats. Average size of coronary small arteries in A was 104 ± 12 μm [range of 83–141 μm intraluminal diameter (ID)], and the average size of coronary small arteries in B was 218 ± 18 μm (range of 162–285 μm ID). Values represent means ± SE.

B_max was achieved at 1 μg of protein per well for coronary artery membranes from both groups of rats. There were no differences in B_max (fmol/mg protein) and K_d (nM) in coronary artery membranes from DOCA-salt rats compared with placebo rats.

Changes in [Ca²⁺]ᵢ, measurement of fura 2. To determine whether alterations in [Ca²⁺]ᵢ responses could explain the impaired contraction to ET-1, fura 2 fluorescence was measured in smooth muscle cells freshly dispersed from coronary small arteries (50–300 μm ID) of placebo and DOCA-salt rats. A typical response to ET-1 (10⁻⁹ M) in a smooth muscle cell from a placebo rat and DOCA-salt rat is shown in Fig. 3, A and B, respectively. ET-1 induced a rapid increase in [Ca²⁺]ᵢ, as indicated by an increased ratio of F₃₄₀ nm to F₃₈₀ nm, followed by a plateau phase that remained above baseline in the smooth muscle cell from a placebo rat. This effect was completely abolished by the selective ETA receptor antagonist A-127722 (30 nM) (data not shown). Conversely, ET-1 (10⁻⁹ M) had no effect on [Ca²⁺]ᵢ in the smooth muscle cell from a DOCA-salt...
rat. In the same cell, KCl (50 mM) produced a rapid and sustained increase in \([\text{Ca}^{2+}]_i\), indicating viability of the cell. A summary of the effect of ET-1 on \([\text{Ca}^{2+}]_i\) in smooth muscle cells from placebo and DOCA-salt rats is shown in Fig. 4. The maximum increase in \([\text{Ca}^{2+}]_i\), as indicated by the change in fura 2 fluorescence, was significantly greater in smooth muscle cells from placebo rats compared with DOCA-salt rats.

DISCUSSION

ET-1 has been shown to contribute to the development of vascular hypertrophy, increased total peripheral resistance, and hypertension in DOCA-salt hypertensive rats (1, 8, 20). In the present study, the concentration of plasma ET-1 was increased in DOCA-salt rats.

Fig. 4. ET-1 (10^{-9} M)-induced change in \([\text{Ca}^{2+}]_i\), as indicated by the change in the ratio F_{340 nm}/F_{380 nm} in single smooth muscle cells freshly dispersed from coronary small arteries (50–300 μm ID) of DOCA-salt hypertensive rats (n = 5), DOCA-salt hypertensive rats (n = 5). F indicates fura 2 fluorescence.

Table 2. B_{max} and K_d of ^{125}I-labeled ET-1 binding in membrane preparations from coronary small arteries of placebo and DOCA-salt rats

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>DOCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>B_{max}, fmol/mg</td>
<td>535 ± 38</td>
<td>477 ± 44</td>
</tr>
<tr>
<td>K_d, nM</td>
<td>0.109 ± 0.03</td>
<td>0.14 ± 0.045</td>
</tr>
</tbody>
</table>

Values represent means ± SE. Coronary small arteries from 5 placebo rats or 5 DOCA-salt rats were pooled for each n, and n = 3 placebo groups and 3 DOCA groups. There were no significant differences. B_{max}, maximum binding; K_d, dissociation constant.

Fig. 3. Agonist-induced changes in intracellular \([\text{Ca}^{2+}]_i\) concentration (F_{340 nm}/F_{380 nm}) in single smooth muscle cells freshly dispersed from coronary small arteries (50–300 μm ID) of normal Sprague-Dawley rats (A) and a DOCA-salt hypertensive rat (B). F indicates fura 2 fluorescence. ET-1 concentration: 10^{-9} M; KCl concentration: 50 mM.
salt rats. In humans, elevated plasma ET-1 concentrations were found to be associated with reduced coronary vasomotor responses (3). Previously, we reported that oral administration of an ETA receptor antagonist in DOCA-salt hypertensive rats partially restored contraction to ET-1 in coronary small arteries, suggesting that ET-1-induced activation of ETA receptors may play an important role in coronary vascular dysfunction associated with DOCA-salt hypertension (9). Others have shown that inhibition of ETA-ETB receptors decreases myocardial hypertrophy and fibrosis in DOCA-salt hypertensive rats (15). Subendocardial arteriolar growth and capillary rarefaction were partially corrected by ETA receptor antagonism in DOCA-salt hypertensive rats (19). Collectively, these findings suggest a role for ET-1 in altering coronary vasculature function in this model of hypertension.

Previously, we evaluated the role of nitric oxide in modulating contraction to ET-1 and found that inhibition of nitric oxide synthase did not restore contraction to ET-1 in coronary small arteries from DOCA-salt rats (9). Therefore, the present study focused on the role of endothelin receptor binding and changes in [Ca^{2+}]. To obtain enough protein to perform these studies, coronary small arteries of a relatively wide size range (50–300 μm ID) were dissected and pooled, with larger vessels providing a greater percentage of the total tissue studied. Initial experiments were designed to confirm that contraction to ET-1 was impaired in vessels throughout this size range. As reported previously, contraction to ET-1 was mediated through ETA receptors in coronary small arteries (150–300 μm ID) from placebo rats and was significantly reduced in DOCA-salt vessels of similar size. Contraction to ET-1 was also decreased in smaller arteries (50–149 μm ID) isolated from DOCA-salt compared with placebo rats. Because functional changes were similar in the range of vessel size studied, this range of vessel internal diameter was used for studies on endothelin receptor binding and changes in [Ca^{2+}].

Endothelin receptor binding was not altered in membranes of coronary small arteries from DOCA-salt rats compared with placebo rats. In the large mesenteric artery bed of DOCA-salt rats, a decrease in endothelin receptor binding, but no change in affinity, has been observed (24). The decreased receptor binding is believed to contribute to the decrease in vascular contraction to ET-1 observed in large mesenteric arteries (24). This effect has been attributed to increased tissue levels of ET-1. Despite our finding that plasma ET-1 levels were increased and the finding of others that ET-1 mRNA expression is increased in the endothelium of large epicardial and intramyocardial coronary arteries in DOCA-salt rats (18), coronary small artery endothelin receptor binding was not reduced. Interestingly, in the renal medulla of DOCA-salt rats, an increase in [125I]ET-1 binding, mediated by an upregulation of ETB receptors, was observed (25). This effect may be an attempt to lower arterial pressure by increasing salt and water excretion via ETB receptor activation. Collectively, these findings indicate that endothelin receptor binding may be increased, decreased, or unchanged depending on the tissue studied and can result in a variety of functional effects.

Because endothelin receptor binding was not altered in coronary small arteries from DOCA-salt rats, the role of changes in [Ca^{2+}] was determined. The ET-1-induced increase in [Ca^{2+}] was absent in coronary small artery smooth muscle cells from DOCA-salt rats compared with placebo rats. This is in agreement with findings in other vascular beds, including the aorta and large mesenteric artery, in which ET-1 induced vascular contraction and increases in cytosolic free Ca^{2+} and inositol phosphate accumulation were reduced in DOCA-salt rats (7, 17, 21). ET-1-induced [Ca^{2+}], responses were also significantly attenuated in cardiomyocytes and fibroblasts of DOCA-salt rats (30). The mechanisms mediating altered calcium handling in DOCA-salt rats is not known, but it has been suggested that increased [Ca^{2+}], observed in DOCA-salt rats may exert a negative feedback effect on ET-1-induced calcium entry (23).

In the DOCA-salt rat model of hypertension, ET-1-induced contraction is attenuated in many vascular beds, including the coronary vascular bed (6, 9, 10, 12, 22, 24). The decreased contraction to ET-1 may be a compensatory response to increases in plasma ET-1, tissue ET-1, and mean arterial pressure. In the peripheral circulation, the reduced contraction may be an important protective mechanism to prevent increases in peripheral vascular resistance. However, in the coronary circulation, appropriate myocardial perfusion requires a balance between vasoconstrictor and vasodilatory systems. Endothelin has been shown to induce uneven flow distribution in the heart (13). The present study, which is the first to evaluate mechanisms mediating altered contraction to ET-1 in coronary small arteries from DOCA-salt hypertensive rats, has shown that the ET-1-induced coronary artery contraction and increase in [Ca^{2+}] are impaired, whereas endothelin receptor binding is not altered. These results suggest endothelin receptor uncoupling from signaling mechanisms and indicate that impaired [Ca^{2+}], signaling contributes to the decrease in ET-1-induced contraction of coronary small arteries in DOCA-salt hypertensive rats.

This work was supported in part by National Heart, Lung, and Blood Institute Grants HL-49924 (to L. C. Fuchs), HL-60653 (to J. S. Pollock), and HL-64776 (to D. M. Pollock); an American Heart Association Established Investigator Award (to L. C. Fuchs); and American Heart Association Scientist Development Grants (to D. M. Pollock and J. S. Pollock).

REFERENCES
3. Cox ID, Botker HE, Bagger JP, Sonne HS, Kristensen BO, and Kaski JC. Elevated endothelin concentrations are associated with reduced coronary vasomotor responses in patients with


