Coronary vascular reactivity is improved by endothelin A receptor blockade in DOCA-salt hypertensive rats

ARARAT D. GIULUMIAN,1 DAVID M. POLLOCK,2 NATA莉E CLARKE, and LESLIE C. FUCHS1

1Department of Pharmacology and Toxicology and 2Department of Physiology and Endocrinology, Vascular Biology Center, Medical College of Georgia, Augusta, Georgia 30912

Giulumian, Ararat D., David M. Pollock, Natalie Clarke, and Leslie C. Fuchs. Coronary vascular reactivity is improved by endothelin A receptor blockade in DOCA-salt hypertensive rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43):R1613–R1618, 1998.—Endothelin-1 (ET-1) is thought to play an important role in the development of deoxycorticosterone acetate (DOCA)-salt hypertension. Because hypertension is associated with an increased incidence of coronary artery disease, this study was designed to determine if coronary vascular contraction to ET-1 is altered in DOCA-salt hypertensive rats and to determine the effect of chronic treatment of DOCA-salt rats with the selective ETA receptor antagonist A-127722. Male Sprague-Dawley rats were divided into four groups: DOCA, Placebo, DOCA + A-127722, and Placebo + A-127722. A-127722 was administered in drinking water at a concentration of 0.8 mg/100 ml. After 3 wk, mean arterial pressure (MAP) was significantly enhanced in DOCA-salt compared with Placebo rats. A-127722 significantly inhibited the increase in MAP. Contractile response to ET-1 (10^-11 to 3 x 10^-8 M) was measured in isolated coronary and mesenteric small arteries (200–300 µm, intraluminal diameter) maintained at a constant intraluminal pressure of 40 mmHg and was significantly impaired in vessels from DOCA-salt compared with Placebo rats. Dose-dependent contractions to KCl were also inhibited in coronary, but only minimally impaired in mesenteric, arteries of DOCA-salt rats. Inhibition of nitric oxide synthesis activity did not restore contraction to ET-1 in coronary small arteries. However contractions to ET-1 were enhanced in mesenteric small arteries. Chronic treatment with A-127722 significantly restored contraction to ET-1 in coronary, but not in mesenteric, arteries of DOCA-salt rats. Because ETα receptor blockade improves the development of hypertension and improves coronary vascular reactivity, these data indicate that ET-1 plays an important role in coronary vascular dysfunction associated with DOCA-salt hypertension.

METHODS

General procedures. Male Sprague-Dawley rats (200 g) (Harlan Laboratories, Indianapolis, IN) were uninephrectomized under methohexital sodium (Brevital) anesthesia. DOCA-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. Rats were further subdivided into two groups that either did or did not receive the selective ETA receptor antagonist A-127722 (24, 30). Comprised with other ETα selective antagonists, A-127722 has the advantages of high potency, oral bioavailability, in vivo efficacy, and extended duration of action (24). A-127722 (8 mg/100 ml) was administered in drinking water. Thus four groups of rats were studied: DOCA, Placebo, DOCA + A-127722, and Placebo + A-127722.

After 3 wk, rats were briefly anesthetized with Brevital for placement of a carotid arterial cannula. Arterial pressure was measured 24 h later in conscious rats via a pressure transducer connected to a MacLab 8e data acquisition system (ADI Instruments, Milford, MA). Rats were then anesthetized with pentobarbital sodium (60 mg/kg ip), and heparin (100 U) was administered via the arterial catheter. A section of the small intestine ~2 cm below the stomach was closed and removed with the mesentery intact for isolation of a third-order branch of the superior mesenteric artery. The heart was removed for dissection of a second-order branch of the left main coronary artery. All tissues were placed in chilled, oxygenated (20% O2, 5% CO2, balance N2) Krebs-Ringer bicarbonate solution (mM composition: 118.3 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, and 11.1 dextrose).
Coronary and mesenteric small arteries were dissected using an Olympus dissection scope. Segments (1–2 mm in length) were mounted in a vessel bath between two glass micropipettes (70 µm in diameter tip) and secured with 10–0 silk ophthalmic suture. The lumen of the vessel was filled with Krebs buffer through the micropipette and maintained at a constant pressure of 40 mmHg. Vessels were monitored under an Olympus inverted light microscope connected to a video monitor. ID was continually measured using a video dimension analyzer (Living Systems Instrumentation, Burlington, VT) and recorded on a Grass polygraph.

Protocol. Oxygenated (20% O2-5% CO2) Krebs solution was maintained at 37°C and circulated continuously through the tissue bath. Vessels removed from rats treated with A-127722 were washed with 1 liter of Krebs solution to assure removal of A-127722. All vessels were allowed to equilibrate for at least 30 min before dose-response curves were performed. Dose-response curves to ET-1 (10⁻¹¹ to 3 × 10⁻⁸ M) were performed in the absence or presence of either A-127722 (30 nM) or N-nitro-L-arginine (L-NNA, 1 mM), an inhibitor of nitric oxide synthase activity. Vessels were pretreated with either A-127722 or L-NNA for 20 min before a dose-response curve to ET-1 was performed. Additionally, a dose-response curve to KCl (25–125 mM) was performed in separate coronary and mesenteric small arteries. Only one experiment was performed per vessel and each experiment was performed only once per rat.

Chemicals. ET-1 (human, porcine), KCl, and L-NNA used in this study were obtained from Sigma Chemical (St. Louis, MO). Brevital (methohexital sodium) was obtained from Lilly (Indianapolis, IN). Nembutal (pentobarbital sodium) and A-127722 were obtained from Abbott Laboratories (Abbott Park, IL). ET-1 was dissolved in 1% bovine serum albumin and diluted with Krebs solution. L-NNA was dissolved in acidic nanopure water and adjusted to a pH of 7.4 with 0.1 N NaOH and diluted in Krebs solution. All other agents were dissolved in nanopure water and diluted in Krebs solution.

Data analysis. ID measurements obtained from coronary and mesenteric vessels were expressed in micrometers. Responses to vasoconstrictor agents were expressed as percent contraction from baseline diameter (in vitro diameter with 40 mmHg intraluminal pressure). 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 from multiple comparisons. The criterion for significance was P < 0.05.

RESULTS

Dose-response curves to ET-1 in coronary small arteries from Placebo and DOCA-salt rats are shown in Fig. 1. ET-1 produced dose-dependent contractions of coronary arteries from Placebo rats. Contraction to ET-1 was significantly impaired in coronary arteries from DOCA-salt compared with Placebo rats. After the dose-response curve to ET-1 was completed, the addition of acetylcholine (10⁻⁵ M) resulted in relaxation of all vessels to baseline, indicating that the endothelium was intact (data not shown). Inhibition of nitric oxide synthase with L-NNA (1 mM) had no significant effect on contraction induced by ET-1 in coronary small arteries from DOCA-salt or Placebo rats. ET-1 produced dose-dependent contractions of mesenteric small arteries from Placebo rats as shown in Fig. 2. Addition of acetylcholine (10⁻⁵ M) at the end of the dose-response curve caused relaxation of all vessels, indicating that the endothelium was intact. Contraction to ET-1 was significantly impaired in mesenteric small arteries from DOCA-salt compared with Placebo rats. The response to ET-1 (10⁻⁸ M) in the presence of L-NNA in mesenteric small arteries from Placebo and DOCA-salt rats is shown in the typical tracing in Fig. 3. Pretreatment of mesenteric small arteries with L-NNA resulted in an initial maximum contraction to ET-1 that was not sustained in vessels from DOCA-salt rats. The maximum contraction was followed by return of vessel diameter to a steady-state level of contraction. Conversely, contraction to ET-1 was sustained in vessels from Placebo rats. The effect of L-NNA on the maximum contraction to each concentration of ET-1 in mesenteric small arteries from Placebo and DOCA-salt rats is summarized in Fig. 2, while the effect of L-NNA on the steady-state response to ET-1 is summarized in Fig. 4. The maximum contraction to ET-1 at concentrations of 3 × 10⁻⁹ and 10⁻⁸ M was largely restored by L-NNA and was not significantly different from the
ET-1 in the concentration range of 10³ to 30 nM A-127722 completely inhibited contraction of small arteries from DOCA-salt and Placebo rats with the steady-state response to ET-1 at 3 µM. L-NNA significantly enhanced contraction observed in L-NNA-treated mesenteric arteries from Placebo rats. L-NNA significantly enhanced the steady-state response to ET-1 at 3 µM, but had no significant effect on the steady-state contraction to ET-1 at 10⁻⁸ M.

In vitro pretreatment of coronary and mesenteric small arteries from DOCA-salt and Placebo rats with 30 nM A-127722 completely inhibited contraction of mesenteric and coronary small arteries induced by ET-1 in the concentration range of 10⁻¹¹ to 10⁻⁸ M in both groups of rats (data not shown). A-127722 had no significant effect on baseline ID. These results indicate that ET-1-induced contraction is mediated by ETₐ receptors in these vessels.

The role of ETₐ receptors in development of hypertension and changes in vascular reactivity observed in DOCA-salt hypertensive rats was determined with chronic oral administration of A-127722. A summary of mean arterial pressure (MAP) and baseline vascular ID (ID at 40 mmHg intraluminal pressure) is shown in Table 1. MAP was significantly higher in DOCA-salt compared with Placebo rats. Treatment orally with A-127722 markedly attenuated the development of hypertension in DOCA-salt rats. However, MAP remained significantly higher in DOCA-salt-treated rats than Placebo rats. A-127722 did not alter MAP in normotensive Placebo rats. Although DOCA-salt hypertensive rats consumed significantly more A-127722 (9 ± 1 mg/day) than normotensive Placebo rats (3 ± 0.1 mg/day), both groups received A-127722 at a concentration high enough to produce maximal inhibition of ETₐ receptors and maintain selectivity (21). The daily intake of A-127722 remained consistent within Placebo and DOCA-salt rats throughout the study. ID in coronary and mesenteric small arteries was not significantly different between groups.

Chronic treatment of DOCA-salt rats with A-127722 significantly enhanced contraction induced by ET-1 in coronary small arteries and had no significant effect on contraction to ET-1 in vessels from Placebo rats (Fig. 5A). However, chronic treatment with A-127722 did not restore contraction to ET-1 in mesenteric arteries from DOCA-salt rats and impaired contraction to the highest doses of ET-1 in mesenteric arteries from Placebo rats (Fig. 5B). The cause of this impairment is unclear. It is unlikely that residual A-127722 was present, because isolated vessels were washed with 1 liter of Krebs buffer, and this impairment was not observed in coronary arteries from A-127722-treated rats. Also, if A-127722 was present, it would be expected to shift the entire dose-response curve, whereas the impairment was significant only at the highest doses of ET-1.

Contraction to KCl was also measured to determine if vascular changes observed in DOCA-salt hypertension were selective for ET-1. KCl-induced contractions of coronary and mesenteric arteries from all four groups of rats are shown in Fig. 6. A and B, respectively. Although contraction to KCl was abolished in coronary arteries from DOCA compared with Placebo rats, contraction to KCl was only moderately inhibited in mesenteric arteries from DOCA compared with Placebo rats. Interestingly, chronic treatment with A-127722 had no effect on contraction to KCl in mesenteric arteries of either DOCA or Placebo rats, but significantly enhanced coronary artery contraction to KCl over the entire dose-response curve in vessels from Placebo rats. Chronic treatment with A-127722 enhanced contraction to KCl in coronary arteries from DOCA-salt rats at the highest dose (125 mM) only.

Table 1. Vascular intraluminal diameter and arterial pressure

<table>
<thead>
<tr>
<th></th>
<th>Coronary ID, µm</th>
<th>Mesenteric ID, µm</th>
<th>MAP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>243 ± 17</td>
<td>239 ± 10</td>
<td>132 ± 3*</td>
</tr>
<tr>
<td>DOCA</td>
<td>286 ± 25</td>
<td>231 ± 10</td>
<td>197 ± 6</td>
</tr>
<tr>
<td>Placebo + A-127722</td>
<td>259 ± 21</td>
<td>250 ± 9</td>
<td>133 ± 3*</td>
</tr>
<tr>
<td>DOCA + A-127722</td>
<td>242 ± 13</td>
<td>257 ± 12</td>
<td>156 ± 8†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. deoxycorticosterone acetate (DOCA); †P < 0.05 vs. Placebo + A-127722.
**DISCUSSION**

DOCA-salt hypertension is characterized by alterations in vascular structure, including significant hypertrophic remodeling of coronary and mesenteric small arteries and alterations in vascular reactivity, including attenuated endothelium-dependent and -independent vasodilatory responses and enhanced contraction to many vasoconstrictor agents (6, 11, 18, 20, 29). However, depressed contraction to ET-1 has been observed in several vascular beds (6, 11, 20, 22). Coronary vascular responsiveness to ET-1 has not been evaluated in this model of hypertension.

These studies demonstrated that ET-1-induced contraction was impaired in both coronary and mesenteric small arteries of DOCA-salt hypertensive rats. One possible mechanism for the impaired contraction to ET-1 would be enhanced production of the endothelium-derived relaxing factor nitric oxide. Release of endothelium-derived relaxing factors has been shown to modulate the response to vasoconstrictor agents in DOCA-salt hypertension (5, 6, 29). In the current study, nitric oxide was found to contribute to the blunted response to ET-1 in mesenteric, but not coronary, arteries. In another study, denudation of the endothelium did not prevent the impaired contraction to ET-1 observed in mesenteric small arteries from DOCA-salt rats, suggesting that endogenous endothelial products do not play a role in attenuating the response to ET-1 (6). These studies may have yielded different results because endothelial denudation removed all endothelium-derived relaxing and contracting factors, whereas L-NNA directly assessed nitric oxide.

Contraction to KCl was slightly reduced in mesenteric small arteries after 3 wk of DOCA-salt treatment. However, the inhibitory effect was minimal compared with that of ET-1. Contraction to KCl was largely inhibited in coronary small arteries of DOCA-salt rats. Previous reports on the response to KCl in vessels from DOCA-salt rats have yielded controversial results. In one study, the KCl-induced change in tension of coronary artery helical strips was slightly enhanced after 6 wk of DOCA-salt treatment (11). Alternatively, the increase in tension in response to depolarization with
KCI was reduced in mesenteric small arteries from DOCA-salt rats (6). After 10 wk of DOCA-salt treatment, contraction to KCl over the concentration range of 30–125 mM was unchanged in large mesenteric arteries (20). The finding that contraction to KCl was largely impaired in coronary small arteries of DOCA-salt rats indicates that impairment of smooth muscle contraction is not selective for ET-1 in this vascular bed. The impaired responses to KCl and ET-1 observed in coronary small arteries may be related to Ca\(^{2+}\) handling, because both of these vasoconstrictor agents have been shown to open voltage-gated Ca\(^{2+}\) channels (12). A previous study suggested that ET-1-induced calcium entry into aortic vascular smooth muscle cells was altered (16). Furthermore, calcium-induced contraction was found to be attenuated in endothelium-denuded mesenteric arteries from DOCA-salt rats (19).

The decreased vascular responsiveness to ET-1 in DOCA-salt rats may be due to downregulation of receptors (22). This effect may be mediated by increased production of ET-1 (4, 14). An increase in ET-1 mRNA expression in the endothelium of large epicardial and intramyocardial coronary arteries of DOCA-salt rats has been observed (15). Also, a reduction in endothelin receptor density was associated with decreased activation of phospholipase C, reduced production of inositol phosphates, and decreased contraction produced by ET-1 in thoracic aorta and large mesenteric arteries of DOCA-salt rats (9, 22). Although a reduced number of endothelin receptors may contribute to the decreased contraction to ET-1, our study indicates that smooth muscle contraction is impaired in coronary arteries of DOCA-salt rats independently of endothelin receptor stimulation. The reduced contraction to ET-1 may be an important protective mechanism to prevent large increases in peripheral vascular resistance in DOCA-salt hypertension. However, it is important to note that, in the coronary circulation, appropriate transmural distribution of myocardial perfusion requires a balance between vasodilatory and vasoconstrictor systems. A large alteration in coronary responsiveness, as observed in DOCA-salt hypertension, could contribute to changes in transmural distribution of myocardial perfusion leading to ischemia.

A role for ET-1 in the development of DOCA-salt hypertension, but not genetic hypertension, has been shown previously (14, 17, 25, 27). Treatment orally for 3 wk with the combined ETA-ETB receptor antagonist bosentan impaired the development of hypertension in DOCA-salt rats (17). Similar results were observed with selective ETA receptor antagonists (8, 23, 27). Treatment of DOCA-salt rats with selective ETA or ETA-ETB receptor antagonists also reduced vascular hypertrophy and remodeling (8, 17). In our experiments, treatment with the selective ETA receptor antagonist A-127722 significantly inhibited blood pressure elevation in DOCA-salt rats, confirming the existence of an endothelin-dependent component in this model of hypertension.

ETA receptors mediated contraction to ET-1 in both coronary and mesenteric small arteries from DOCA-salt and Placebo rats. Chronic ETA receptor antagonism in DOCA-salt rats partially restored reactivity to ET-1 in isolated small coronary arteries. This effect was specific for DOCA-salt rats, because enhanced contraction to ET-1 was not observed in Placebo rats treated with A-127722. In humans with essential hypertension, the blunted contraction to ET-1 observed in small arteries was normalized by antihypertensive treatment (26). We can speculate that the improved contractile function of the small coronary arteries from A-127722-treated DOCA-salt rats may be due to improving development of hypertension or to changes in ET receptors or second messenger systems due to chronic treatment with the ETA receptor antagonist. Although contraction to the highest dose of KCl was enhanced in coronary small arteries of DOCA-salt rats treated with A-127722, a large increase in contraction to KCl was also observed in coronary small arteries from Placebo rats, indicating that this effect is not related to impeding development of hypertension. Previously, we demonstrated that contraction to the \(\alpha\)-adrenoceptor agonist phenylephrine, but not ET-1, was enhanced in coronary arteries from spontaneously hypertensive rats compared with normotensive Wistar-Kyoto rats (7). Interestingly, treatment with hydralazine lowered arterial pressure in spontaneously hypertensive rats, but did not alter contraction to phenylephrine or ET-1. Collectively, these results indicate that alterations in ET-1-induced contraction of coronary arteries are dependent on the model of hypertension and that changes in coronary vascular reactivity associated with treatment of hypertension may be selective for the model and antihypertensive agent administered.

Improvement in contraction to ET-1 was specific for coronary arteries and was not observed in the mesenteric vascular bed. Heterogeneity in smooth muscle and endothelial cell shape and function and vascular reactivity among different vascular beds have been reported in many studies (1, 3, 8). Although the impaired contraction to ET-1 was observed in both coronary and mesenteric small arteries in DOCA-salt hypertension, the mechanisms mediating this effect are clearly heterogeneous. Nitric oxide was found to play a role in modulating the response to ET-1 in the mesenteric, but not coronary, small artery. The results of this study are consistent with the possibility that alterations in Ca\(^{2+}\)-channel-mediated smooth muscle cell contraction may contribute to the observed responses. However, vascular contraction induced by depolarization was impaired to a much larger extent in coronary than mesenteric small arteries. In summary, oral administration of A-127722 enhances coronary vascular reactivity to ET-1 in a manner that may improve coronary function in the DOCA-salt model of hypertension.

These experiments were supported in part by National Heart, Lung, and Blood Institute Grant HL-49924.

Address for reprint requests: L. C. Fuchs, Vascular Biology Center, Medical College of Georgia, Augusta, GA 30912.

Received 14 October 1997; accepted in final form 23 February 1998.
REFERENCES