ET\textsubscript{A} Receptor Mediates Altered Leukocyte-Endothelial Cell Interaction and Adhesion Molecules Expression in DOCA–Salt Rats

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Abstract—Leukocyte adhesion to endothelial cells plays a key role in inflammatory processes associated with end-organ injury. Endothelin-1 (ET-1), which stimulates inflammatory processes, contributes to cardiovascular damage in deoxycorticosterone (DOCA)–salt hypertension. We investigated whether ET\textsubscript{A} receptor blockade modulates in vivo leukocyte–endothelial cell interactions and expression of cell adhesion molecules (CAM) involved in these processes. DOCA–salt and control uninephrectomized rats were treated with the ET\textsubscript{A} antagonist BMS182874 (40 mg/kg per day) or vehicle. Analysis of CAMs expression by reverse transcription-polymerase chain reaction and immunohistochemistry showed increased cardiac platelet selectin (P-selectin), detected mainly in endothelial cells, and vascular cell adhesion molecule-1 (VCAM-1), but not intercellular adhesion molecule-1 (ICAM-1), in DOCA–salt rats. Cardiac expression of endothelial selectin (E-selectin) was decreased, whereas immunoreactivity to ED-1 and myeloperoxidase (MPO) activity, markers of macrophage and leukocyte infiltration, respectively, were increased in DOCA-salt. Leukocyte–endothelial cell interaction, functionally assessed in venules of internal spermatic fascia by intravital microscopy, was significantly altered in DOCA–salt rats as evidenced by increased leukocyte adhesion and decreased rolling. BMS182874 treatment normalized leukocyte–endothelium interactions, decreased cardiac VCAM-1 expression in DOCA and control groups, and had no effects on ICAM-1 expression. BMS182874 also increased E-selectin and abolished P-selectin expression in DOCA-salt, but not in control rats. The ET\textsubscript{A} antagonist reduced cardiac ED-1 content and MPO activity and prevented cardiac damage in DOCA–salt rats. These data indicate that ET-1 participates, via activation of ET\textsubscript{A} receptors, in altered leukocyte–endothelial cell interactions in DOCA–salt rats, possibly by modulating expression of CAMs, and that the inflammatory status is associated with cardiac damage in mineralocorticoid hypertension. (Hypertension. 2004;43:872-879.)

Key Words: endothelin • deoxycorticosterone • arterial • hypertension • leukocytes • cell adhesion molecules

Endothelin-1 (ET-1), a peptide initially characterized as a potent vasoconstrictor, is overexpressed in the vasculature of different models of hypertension, such as deoxycorticosterone (DOCA)–salt rats,\textsuperscript{1} the genetic salt-sensitive Dahl\textsuperscript{2} and Sabra\textsuperscript{3} rats, and aldosterone-treated rats,\textsuperscript{4} as well as in patients with moderate to severe hypertension.\textsuperscript{5} ET-1 stimulates expression of proinflammatory molecules and is considered an important mediator of chronic inflammation in the vascular wall of hypertensive animals.\textsuperscript{6,7} Because vascular inflammation is associated with development of end-organ damage, ET-1–induced inflammatory processes may contribute to cardiovascular injury in hypertension.\textsuperscript{7}

Leukocyte adhesion, resulting from the expression of cell surface adhesion molecules (CAM), is a key factor in the pathogenesis of vascular dysfunction and tissue injury.\textsuperscript{8} Different families of CAMs mediate interactions between circulating leukocytes and the blood vessel wall, such as: (1) selectins expressed on leukocytes (L-selectin), endothelial cells (E-selectin), and platelets (P-selectin); (2) integrins; and (3) molecules of the immunoglobulin gene superfamily, intercellular cell adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1).\textsuperscript{5} Expression of many of these CAMs and cell migration into the vessel wall is upregulated in different forms of hypertension.\textsuperscript{9–13} In double-transgenic rats (dTGR) harboring the human renin and angiotensinogen genes, ICAM-1 and VCAM-1 are expressed on the endothelial cell surface of cardiac vessels, whereas the respective ligands are expressed on circulating leukocytes.\textsuperscript{9} Likewise, increased CAM expression has been described in vessels of other models of hypertension with
high renin levels.\textsuperscript{10,11} Spontaneously hypertensive rats and aldosterone-treated rats exhibit increased expression of endothelial ICAM-1 and macrophage chemoattractant protein (MCP-1), as well as an increased number of ED-1-positive cells (macrophages) within the vascular wall.\textsuperscript{12,13} Expression of many inflammatory mediators (cytokines, interleukins, chemokines, and CAMs) is regulated by oxidative stress-sensitive transcription factors such as nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1).\textsuperscript{3,14} The presence of ET-1-dependent oxidative stress and inflammatory response have been previously described in DOCA-salt rats\textsuperscript{15-18} and may be involved in cardiovascular remodeling and end-organ damage in this model of hypertension. However, little is known about the role of ET-1 in in vivo leukocyte–endothelial cells interactions and in the cardiac expression of CAMs in DOCA-salt rats.

In view of these observations, the present study was designed to assess: (1) in vivo alterations in leukocyte–endothelial cells interactions in DOCA-salt rats; (2) changes in cardiac expression of CAMs associated with leukocyte–endothelial cells interaction; (3) role of ET-1 in these processes; and (4) existence of a correlation between expression of inflammatory markers and cardiac injury in DOCA-salt rats. This is of interest because leukocyte recruitment, which contributes to vascular lesions that occur in hypertension and myocardial ischemia–reperfusion injury, might be prevented using ET receptor antagonists.

Methods

Animal Experiments

Experimental protocols followed standards and policies of the University of Sao Paulo’s Animal Care and Use Committee. Male Wistar rats had ad libitum access to standard laboratory rat chow and water and were housed individually in a room with a constant temperature (24°C) and a 12-hour/12-hour light/dark cycle. DOCA-salt hypertension was induced as previously described\textsuperscript{19} and, simultaneously, rats received either the ET\textsubscript{A} antagonist BMS182874 (40 mg/kg, SC) and the internal spermatic fascia of the scrotal chamber was exteriorized for microscopic examination in vivo in situ. Vessels selected for study were third-order venules, with diameters ranging from 16 to 20 μm; interaction of leukocytes with the luminal surface of the venular endothelium was studied in a 100-mm length segment of the vessel. Rolling leukocytes (rollers) were defined as those white blood cells that moved at a velocity less than that of erythrocytes in the same stream. These leukocytes moved sufficiently slowly to be individually visible and were counted in 10-minute periods.\textsuperscript{20} Leukocytes adhering to the venular endothelium (stickers), ie, those that remained stationary for at least 30 seconds, were also quantified in the same 10-minute periods and were expressed as the number per 100-mm length of venule.

Centerline blood cell velocity (RBCV) was also measured in venules of the internal spermatic fascia with an optical Doppler velocimeter. Venular blood flow was calculated from the product of mean centerline velocity (Vmean) and microvascular cross-sectional area, with cylindrical geometry assumed. Venular wall shear rate (γ) was calculated from the Newtonian definition: \( γ = 8\times V\text{mean}/Dv \).

Cardiac Adhesion Molecule mRNA

Reverse-transcription polymerase chain reaction (RT-PCR) analysis, used to evaluate adhesion molecules gene expression, was performed as described.\textsuperscript{18} Total cellular RNA was isolated from left ventricles. After DNA contaminant digestion, first-strand complementary DNA (cDNA) was synthesized using 2 μg total RNA. cDNA was amplified using the following set of primers (5’-3’): VCAM, sense = AAGGGGCTAC ATCCACA CTCG, antisense = ACCGGTG- CATGTGACAGTGAC; ICAM, sense = CTTCTTGCGA AGACGAGAAC, antisense = ACTCGCTTCTGGAGACGAAAT; E-selectin, sense = GGGATTTAGG GACAGAAGAGAGT, antisense = TCTTTG TCTACAGACATC; P-selectin, sense = TAATCCCCCG CAGTGTAAAG, antisense = AGGTGCGGAA GGACGAAAT; and GAPDH, sense = TTATGATGACA TCAAGAAAGGTG, antisense = CACCC ACCTG TTGCTGTGTA. The following conditions were used: an initial denaturing cycle at 94°C for 3 minutes, subsequent cycles with denaturation at 94°C for 30 seconds, annealing at 62°C (ICAM-1, P-selectin, E-selectin, and GAPDH) or 64°C (VCAM-1) for 45 seconds, and extension at 72°C for 45 seconds. Amplification cycles were: VCAM-1, 34; ICAM-1, 38; P-selectin, 38; E-selectin, 32; and GAPDH, 24. Signals were expressed relative to the GAPDH intensity (used as an internal control) in each coamplified sample. RNA isolated from the internal spermatic fascia of rats locally treated with tumor necrosis factor-α (TNFα) (0.5 mg, subcutaneous) was used as a positive control.

Immunohistochemistry

Frozen left ventricles were cryosectioned (7-μm thickness) and fixed with cold acetone for 10 minutes. For direct immunohistochemistry, sections were incubated with 3% H\textsubscript{2}O\textsubscript{2} and a Pierce solution to block endogenous peroxidase and biotin, respectively, followed by an overnight incubation (humidified box, 4°C) with a biotinylated anti-rat VCAM-1 monoclonal antibody (BD Pharmingen) or biotin-conjugated anti-rat ICAM-1 monoclonal antibody (Seikagaku). For P-selectin, an indirect technique and an additional blockade of nonspecific binding sites with 50% rabbit serum were performed before incubation with the primary anti-human P-selectin polyclonal antibody (1:50; BD Pharmingen). Sections were incubated for 60 minutes with a secondary biotin-conjugated anti-rabbit antibody (1:1000 in 2% horse serum; Rockland) and with streptavidin conjugated to horseradish peroxidase (Vector Labs). Color was developed by the addition of DAB (Sigma). Sections were lightly stained in hematoxylin, dehydrated with alcohol and xylene, and scored by an independent observer unaware of the groups and treatments of the rats. Vascular endothelium of the internal spermatic fascia from TNFα-treated animals was used as positive control.

For ED-1, ventricles were fixed in Methacarn (60% methanol, 30% chloroform, and 10% acetic acid), and paraffin-embedded longitudinal sections (7 μm) were heated in 0.01 mol/L citrate buffer (pH 6.0) and sequentially incubated with primary monoclonal antibodies against rat monocytes/macrophages (1:500 dilution; Serotec) and with a biotin-conjugated secondary anti-mouse antibody (1:1000, Vector Labs). To evaluate background reaction, procedures were also performed in sections incubated only with the secondary antibody (indirect technique) or in the absence of antibodies (direct technique).

Histopathological Analysis

Left ventricular myocardium was fixed for 24 hours with 4% formaldehyde solution, dehydrated, embedded in paraffin, and sectioned transversely (4 μm). Sections were stained with hematoxylin and Sirius red and scored for vascular damage and collagen deposition.
Determination of Myeloperoxidase Activity
Myeloperoxidase (MPO) activity was performed as described.24 Left ventricles were homogenized in 50 mmol/L potassium phosphate buffer (pH 6.0) containing 0.05% hexadecyltrimethylammonium bromide (HTAB) (50 mg tissue/mL). The suspension was centrifuged (12,000g, 5 minutes, 4°C) and supernatant was heated (2 hours, 60°C) to inactivate peroxidase activity. Tissue-associated MPO activity was assessed by measuring H2O2-dependent oxidation of O-dianisidine dihydrochloride. Changes in sample absorbance were measured in 460 nm and recorded for 2 minutes.

Data Analysis
Results are expressed as means±SEM; n indicates the number of animals. Statistical significance was evaluated by ANOVA or Student t test, as appropriate; P<0.05 was considered significant.

Results
Blood Pressure Measurement
SBP increased progressively after DOCA treatment and salt loading. At 5 weeks of treatment, SBP (mm Hg) in DOCA–salt rats (210±9; n=20; P<0.05) was higher than in UnINX (118±3; n=15). Treatment with the ETA antagonist BMS182874 significantly lowered SBP in DOCA–salt rats (170±4; n=22, P<0.05), but not in UnINX (120±3; n=15), compared with vehicle-treated groups.

Leukocyte–Endothelial Interaction
An increased number of adherent leukocytes and decreased number of rollers was observed in the venules of the internal spermatic fascia from DOCA–salt rats versus UnINX (Table 1). These differences were not related to altered number of circulating cells, because total counts of neutrophils/leukocytes were similar between groups. Venules of the internal spermatic fascia from DOCA–salt and UnINX rats also had a similar centerline RBCV (2.1±0.2 versus 1.9±0.1 mm/s) and shear rate (702±66 versus 695±29 s⁻¹). Changes in leukocyte–endothelial cell interaction in DOCA–salt rats were corrected by BMS182874 treatment (Table 1).

Gene Expression of Adhesion Molecules
Figure 1 shows CAM gene expression in left ventricular myocardium from DOCA–salt and UnINX rats. Increased cardiac VCAM-1 expression was observed in DOCA–salt (1.5±0.1; P<0.05) versus UnINX (1.1±0.1) rats (Figure 1A). Treatment with BMS182874 decreased VCAM-1 expression in both groups and abolished the differences between DOCA–salt (0.2±0.05) and UnINX (0.1±0.02) rats. Cardiac expression of ICAM-1 was not different between DOCA–salt (1.0±0.2) and UnINX rats (0.8±0.2) (Figure 1B). BMS182874 had no effects on ICAM expression (0.7±0.2 DOCA versus UnINX 0.9±0.1). E-selectin expression was decreased in DOCA–salt (0.2±0.07) compared with UnINX (0.9±0.1) and BMS182874 treatment increased E-selectin expression in DOCA–salt (0.43±0.1) without affecting that in UnINX (0.8±0.03) (Figure 1C). A very slight expression of P-selectin was observed in UnINX (0.04±0.03), whereas high expression was detected in DOCA–salt (0.2±0.1). Treatment with the ETA antagonist abolished P-selectin expression in both groups (Figure 1D).

Immunostaining of Cell Adhesion Molecules
Representative photomicrographs of CAM immunostaining in cardiac sections from DOCA–salt and UnINX rats and scores are shown in Figure 2 and Table 2, respectively. Compared with UnINX (Figure 2A, 2B), VCAM-1 expression was more evident in the endothelium of arteries (Figure 2C) and venules (Figure 2D) from DOCA–salt. BMS182874 treatment abolished the higher immunostaining for VCAM-1 in DOCA–salt sections (Figure 2E, 2F). No changes in ICAM-1 staining were observed, and treatment with BMS182874 had no effect on ICAM-1 expression in DOCA–salt or UnINX rats (Table 2). No staining for P-selectin was observed in sections from UnINX animals (Figure 3A, 3B). P-selectin was weakly present in the endothelium of arteries (Figure 3C) and venules (Figure 3D), as well as in vascular smooth muscle and cardiac cells (Figure 3C) from DOCA–salt rats. No immunoreactivity to P-selectin was noted in sections from BMS182874-treated DOCA–salt rats (Figure 3E, 3F).

Macrophage Infiltration
Figure 4 shows macrophage infiltration in left venules from DOCA–salt rats. No staining was detected in sections from UnINX or BMS182874-treated DOCA–salt rats.

Myeloperoxidase Activity
MPO activity was detected in all experimental groups (Figure 5). In hearts from DOCA–salt rats, MPO activity was significantly increased compared with UnINX (7.3±1.2 versus 4.5±0.8 U/g tissue, respectively). BMS182874 treatment decreased MPO activity only in DOCA–salt rats (1.6±0.3 U/g tissue) without affecting that in UnINX rats (4.4±0.5 U/g tissue).

Histopathological Analysis
Compared with sections from UnINX (Figure 6A, 6B), left venules of DOCA–salt rats revealed severe damage characterized by intense collagen deposition, fibrinoid necrosis (Figure 6C; Table 3), and thickening of small arteries (Figure 6D). Treatment of DOCA–salt rats with BMS182874 alleviated these changes (Figure 6E, 6F).

Discussion
Cell adhesion molecules play a role not only in normal physiological processes but also in the inflammatory response that contributes to vascular dysfunction and target organ damage in hypertension.8 We assessed the inflammatory status in DOCA–salt rats by evaluating in vivo leukocyte behavior and cardiac expression of CAMs. The involvement of ET-1 on these

| Table 1. Effect of BMS Treatment on Leukocyte–Endothelial Cell Interactions in DOCA–Salt and UniNX Rats |
|-----------------|-----------------|-----------------|
| Group           | Rollers         | Stickers        |
| UniNX           | 209±6 (6)       | 5±1 (6)         |
| DOCA–Salt       | 100±16* (8)     | 16±3* (8)       |
| UniNX BMS 182874 | 185±12 (4)     | 4±2 (4)         |
| DOCA–Salt BMS 182874 | 197±15 (6) | 4±2 (6)         |

Results are means±SEM. Number of animals is shown in parentheses. *P<0.05 vs UnINX.
processes was evaluated by treating rats either with the ET\(_A\) receptor antagonist BMS182874 or with vehicle. In this study, ET\(_A\) blockade corrected the altered leukocyte–endothelial cells interactions (rolling and adhesion) in DOCA–salt rats, at least in part by normalizing the expression of specific CAMs, and the cardiac inflammatory status correlated with structural changes in this hypertensive model.

The multistep process of leukocyte recruitment is part of a highly reproducible vascular response to inflammation and involves coordinated and regulated expression of structurally

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**Figure 1.** Representative RT-PCR products of 2 \(\mu\)g total RNA extracted from hearts of DOCA–salt and UniNX rats. Bar graphs show the relative optical density values for VCAM-1 (A), ICAM-1 (B), P-selectin (C), and E-selectin (D) in the different groups. Values were normalized by the corresponding RT-PCR products for GAPDH. Results are mean±SEM (n=4 to 6). *P<0.05 versus UniNX. **P<0.05 versus BMS182874-treated DOCA–salt rats.
and functionally distinct families of CAMs. Sequential events in leukocyte–endothelial cell interactions involve rolling, adhesion, and migration of leukocytes. Selectins mediate leukocyte rolling on endothelial cells, and their expression is stimulated by cytokines and oxidative stress. Whereas P-selectins are stored in granules in platelets and endothelial cells from where they are mobilized to the cell surface, expression of E-selectins on the cell surface of endothelial cells occurs on stimulated-protein synthesis. After the selectin-mediated initial and low-affinity leukocyte binding to endothelial cells, further leukocyte activation occurs, resulting in subsequent firm adhesion and transendothelial migration of leukocytes, processes mediated by ICAM-1 and VCAM-1. These CAMs, expressed on many cell types, have their expression positively regulated by cytokines and mediate adhesion of lymphocytes and monocytes to endothelial cells in all segments of the vasculature, especially in post-capillary venules. CAMs of the integrin family, expressed in eosinophils, lymphocytes, monocytes, and smooth muscle cells, also participate in adhesion and migration processes and bind either to molecules of the immunoglobulin family or to a variety of large extracellular matrix proteins (fibronectin, fibrinogen, and so on). The interaction between integrins and extracellular matrix proteins, which is implicated in cytoskeletal reorganization and cell motility, may be associated with rearrangement of smooth muscle cells, increased deposition of extracellular matrix components, changes in cell–cell and cell–matrix interactions, and growth an remodeling.

Altered leukocyte adhesion to endothelial cells has been reported in different models of hypertension and seems to be associated with changes in CAMs expression on endothelial cells, leukocytes, or both. In our study, the initial low-affinity leukocyte–endothelial cell interaction was attenuated in DOCA–salt rats, whereas enhanced leukocyte adhesion was observed. Decreased number of rollers may result from decreased number of leukocytes in the circulation or changes in blood flow/shear stress. However, DOCA–salt and UniNX rats displayed similar numbers of circulating leukocytes and no differences were observed in centerline RBCV or shear rate. It is possible that the subnormal leukocyte rolling is a counter-regulatory mechanism to minimize cell adhesion/migration and injury resulting from the inflammatory response. Of CAMs involved in leukocyte rolling, E-selectin expression was decreased in DOCA–salt hearts, which may explain the functional results. However, cardiac P-selectin expression was increased in these animals, which may represent a homeostatic mechanism.

In contrast to the abnormal leukocyte rolling behavior, DOCA–salt rats displayed increased adhesion, associated with increased cardiac VCAM-1 expression, but no changes in ICAM-1 levels. Increased expression of CAMs, involved in recruitment of circulating leukocytes to sites of inflammation in the vascular wall, and other inflammatory markers is often noted in small arteries from hypertensive animals.

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**Table 2. Scores for CAM Immunostaining**

<table>
<thead>
<tr>
<th>Group</th>
<th>VCAM-1</th>
<th>P-Selectin</th>
<th>ICAM-1</th>
</tr>
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<tbody>
<tr>
<td>UniNX</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>CM</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>A</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td>+</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>VSM</td>
<td>+</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>DOCA–salt</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>CM</td>
<td>–</td>
<td>+</td>
<td></td>
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<td>V</td>
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<td>A</td>
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</tr>
<tr>
<td>VSM</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>DOCA–salt BMS 182874</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>CM</td>
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<td>V</td>
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<td>A</td>
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<td>–</td>
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</tr>
<tr>
<td>VSM</td>
<td>–</td>
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<td>+</td>
</tr>
</tbody>
</table>

Scores: – indicates no staining; +, faint brown; ++, moderate brown; ++++, intense brown.

CM indicates cardiac muscle; V, endothelium in veins; A, endothelium in arteries; VSM, vascular smooth muscle cells.

N=3 to 5 animals in each group.
and has been invoked to explain microvascular dysfunction and tissue injury seen in hypertension.

MPO activity, used as an index of leukocyte infiltration, and macrophage infiltration were increased in DOCA-salt hearts, suggesting that changes in leukocyte-adhesion ob-

served in internal spermatic fascia may be representative of the cardiac circulation. Increased MPO activity was found in isolated rabbit hearts on infusion of ET-133 and, in our study, increased cardiac MPO activity in DOCA–salt rats was reversed by the ET A antagonist BMS182874.

Two transcription factors, NF-kB and AP-1, regulate endothelial CAM expression.8,34,35 Binding sites for NF-kB have been identified in the promoter regions of the VCAM-1 and ICAM-1 genes, whereas a binding site for AP-1 has been

Figure 3. Representative immunohistochemical photomicro-
graphs of P-selectin in left ventricle sections of UniNX (A and B), DOCA-salt (C and D), and BMS182874-treated DOCA-salt rats (E and F). Arrows indicate increased expression of P-selectin. Magnification level was ×60.

Figure 4. Representative immunohistochemical photomicro-
graphs of ED-1-positive cells in left ventricle sections of UniNX (A), DOCA-salt (B), and BMS182874-treated DOCA-salt rats (C). Arrows indicate ED-1-positive cells. N=4 to 5 animals in each group. Magnification level was ×10.

Figure 5. Bar graphs show cardiac myeloperoxidase activity in DOCA-salt and UniNX rats treated with vehicle or BMS182874 (n=4 to 7 animals in each group). Values are means±SEM. *P<0.05 versus UniNX. **P<0.05 versus DOCA-salt rats.

Figure 6. Representative light micrographs of left ventricle sec-
tions, stained with the Picro-Sirius technique, from UniNX (A and B), DOCA-salt (C and D), and BMS182874-treated DOCA-salt rats (E and F). Arrows and circles indicate collagen deposition in the cardiac and vascular interstitium, respectively.
localized on the promoter region of the ICAM-1 gene. Selective activation of these transcription factors and differential modulation of inflammatory-related gene expression may explain the selective modulation of VCAM-1 expression.

ET-1 plays an important role in the pathogenesis of salt-sensitive hypertension and in the associated cardiovascular damage. \(^1\) A relationship between ET-1 and inflammatory process in hypertension was first recognized in the dTGR model, in which the ET\(_A\)/ET\(_B\) receptor antagonist bosentan inhibited the increased cardiac NF-\(\kappa\)B activity, perivascular monocyte/macrophage infiltration, and enhanced cardiac CAMs expression. In DOCA–salt hypertensive rats, increased NF-\(\kappa\)B activation has been reported, and ET\(_A\) receptor-dependent activity of this transcription factor was demonstrated in hearts from these animals. \(^2\)

Altered leukocyte–endothelial cells interactions in venules of DOCA–salt rats as well as altered expression of CAMs were restored by BMS182874 treatment, which suggests a role for the ET-1/ET\(_A\) pathway in these processes. In vivo and in vitro studies with antibodies against CAMs have also demonstrated that ET-1 stimulates leukocyte rolling and adhesion. \(^3\)

Mechanisms whereby ET\(_A\) receptor blockade prevents changes in CAM expression are currently unclear, particularly because there is little evidence for the presence of ET\(_A\) receptors on endothelial cells. ET-1 alone did not induce VCAM-1 expression in human vascular endothelial cells. However, inhibition of endogenous nitric oxide (NO) enhanced surface VCAM-1 expression stimulated by ET-1, indicating that stimulation of an ET-1 endothelial receptor results in the production of NO, suppressing the expression of VCAM-1. \(^4\)

Our findings may result from paracrine regulation of NO synthesis in endothelial cells and an indirect modulation of CAM expression. \(^5\) Long-term treatment with ET\(_A\) receptor antagonists normalizes impaired NO-mediated endothelium-dependent relaxation and increases NOS activity and plasma nitrate levels, a marker of NO activity in vivo. \(^6\)

Another potential mechanism may be a reduction of oxidative stress in the smooth muscle cells leading to diminished NF-\(\kappa\)B activation and decreased inflammatory mediators. Indeed, we have recently shown that DOCA–salt rats display increased ROS generation in the vasculature, which can be prevented by ET\(_A\) blockade. \(^7\)

Importantly, correction of altered in vivo leukocyte behavior and expression of CAMs by ET\(_A\) blockade was accompanied by reduction in cardiac structural damage (intense collagen deposition, fibrinoid necrosis, and vascular hypertrophy), which suggests a relationship between the inflammatory response and the structural changes. We have not used another antihypertensive agent to examine the effect of BP-lowering on the expression of CAMs. However, it is unlikely that normalization of the observed changes was caused by the antihypertensive effects of BMS182874, because BP was only modestly reduced by treatment. In support of this, a previous study demonstrated that ET-1-mediated effects on vascular structure may be pressure-independent. \(^8\)

In summary, the ET-1/ET\(_A\) receptor pathway is involved in the increased migration of leukocytes and in the increased expression of VCAM-1 and P-selectin, markers of inflammation, in DOCA–salt hypertension. These findings suggest that ET\(_A\) antagonism ameliorates cardiac damage partially by correction of altered leukocyte–endothelial cell interaction and via inhibition of mechanisms mediating CAMs expression.

**Perspectives**

The reduction of inflammatory process, normalization of leukocyte–endothelial cell interaction, and amelioration of cardiac lesions by blockade of ET\(_A\) receptors in DOCA–salt rats support an important role for ET-1 in end-organ injury in mineralocorticoid hypertension. Because inflammatory processes, which contribute to cardiovascular lesions in hypertension and myocardial ischemia–reperfusion injury, might be prevented using ET receptor antagonists, these data provide a rationale for the use of ET\(_A\) receptor blockade in some forms of human hypertension.

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