Estrogens Inhibit Angiotensin II–Induced Leukocyte–Endothelial Cell Interactions In Vivo via Rapid Endothelial Nitric Oxide Synthase and Cyclooxygenase Activation

Ángeles Álvarez, Carlos Hermenegildo, Andrew C. Issekutz, Juan V. Esplugues, Maria-Jesus Sanz

Abstract—Angiotensin II (Ang II) may be a key molecule in the development of atherosclerosis. Because the incidence of coronary atherosclerosis in premenopausal women is lower than that observed in men or postmenopausal women, we have investigated the effect of estrogens on Ang II–induced leukocyte recruitment in vivo using intravital microscopy in the rat mesenteric microcirculation. Superfusion for 60 minutes with Ang II induced a significant increase in leukocyte rolling flux, adhesion, and emigration. Administration of 17-β-estradiol (17-β-E) after 30 minutes of Ang II superfusion produced a reduction of these leukocyte responses by 55.1%, 72.7%, and 70.9%, respectively, an additional 30 minutes later. The effect observed with 17-β-E was receptor-mediated and specific. 17-β-E superfusion did not modify either L-NAME or indomethacin-induced leukocyte responses. Inhibitory responses caused by 17-β-E were not altered by either 7-nitroindazole or actinomycin D cosuperfusion. Stimulation of endothelial cells with 17-β-E caused a rapid and dose-dependent release of prostacyclin. Finally, tamoxifen or ICI 182,780 administration provoked a significant increase in leukocyte–endothelial cell interactions 90 minutes later, which were significantly attenuated by systemic preadministration with an Ang II AT1 receptor antagonist. Tamoxifen-induced leukocyte responses were also reduced by systemic pretreatment with an anti–P-selectin mAb and an anti–CD18 mAb. Hence, the antiatherogenic effects of estrogens may be mediated by inhibition of Ang II–induced leukocyte recruitment through endothelial NO and prostacyclin release. Furthermore, scarcity of estrogens resulted in decreased levels of vasodilators and the exposure of the endothelium to the deleterious action of Ang II, which may explain the higher incidence of coronary atherosclerosis in men and postmenopausal women. (Circ Res. 2002;91:lll–lll.)

Key Words: leukocytes ■ endothelium ■ cell adhesion molecules ■ intravital microscopy ■ prostacyclin

Atherosclerosis is the main contributor to the pathogenesis of myocardial and cerebral infarction, gangrene, and loss of function in the extremities. This process bears several histopathologic similarities to chronic inflammation. The early atherosclerotic lesion involves an inflammatory response consisting of intimal accumulation of T lymphocytes and lipid-laden macrophages, which occurs continuously throughout the entire atherogenic process.1,2 The vascular endothelium is a major controller of leukocyte traffic between the blood stream, arterial intima, and extravascular space.3 In this context, the migration of leukocytes from the blood to sites of extravascular injury in response to locally produced stimuli is mediated through the interaction of different adhesive receptors present on leukocyte cell surface with their respective counterreceptors on the endothelial cell. This multistep process is initiated by the tethering of leukocytes to the endothelium, followed by leukocyte rolling, leading ultimately to firm leukocyte adhesion to and subsequent transmigration through the vascular endothelium.4 Angiotensin II (Ang II) is the main effector peptide of the renin-angiotensin system, and in addition to its role as a potent vasoconstrictor and blood pressure and fluid homeostasis regulator, it has been shown to exert proinflammatory activity. Ang II receptors have been demonstrated on human monocytes, and Ang II is capable of promoting monocyte adhesion and activation in vitro.5,6 This may be relevant, because hypertension is associated with migration of monocytes into the vessel wall, a critical event leading to the development of the atherosclerotic lesion, which can be attenuated by angiotensin-converting enzyme inhibition or by pretreatment with an Ang II AT1 receptor antagonist.7–9 Interestingly, we have recently revealed that Ang II shows proinflammatory activity in vivo at subvasoconstrictor doses. In particular, it induces leukocyte trafficking into the rat
mesenteric microvasculature through endothelial P-selectin upregulation in the vessel wall, an effect that is primarily mediated via an Ang II AT₁ receptor interaction. Therefore, Ang II might be a stimulus for the subendothelial infiltration of leukocytes observed in hypertension and atherosclerosis.

The incidence of coronary atherosclerosis in premenopausal women is half that observed in men of the same age. In contrast, postmenopausal women do not exhibit similar protection. An abundance of epidemiological data supports a role for estrogens in this atheroprotective effect, leading to recommendations for their widespread use in postmenopausal replacement therapy. However, the mechanism whereby this protection is mediated remains obscure. An abundance of epidemiological data supports a role for estrogens in this atheroprotective effect, leading to recommendations for their widespread use in postmenopausal replacement therapy. However, the mechanism whereby this protection is mediated remains obscure. In this context, avoiding the proinflammatory activity of Ang II may constitute a plausible explanation for the effects ascribed to estrogens. Therefore, in the present study, we have investigated whether 17-β-estradiol (17-β-E) can reduce or prevent the leukocyte–endothelial cell interactions induced by Ang II in vivo and the mechanism involved in this inhibitory effect. Finally, we have also carried out a series of experiments to explore why reduced levels of these hormones, or their absence, can provoke the formation of the atherosclerotic lesion.

Materials and Methods

Intravital Microscopy

The details of the experimental preparation have been described previously. Briefly, male Sprague-Dawley rats, 200 to 250 g, were anesthetized with sodium pentobarbital (Sigma Química, 65 mg/kg IP), and the trachea, right jugular vein, and carotid artery were cannulated. After a midline abdominal incision was made, a segment of the midjejunum was exteriorized and placed over an optically clear viewing pedestal maintained at 37°C, which facilitated tissue...
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Leukocyte Rolling Velocity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time, Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Ang II</td>
<td>105.3±11.0</td>
</tr>
<tr>
<td>Ang II+17-β-E, 1 nmol/L</td>
<td>101.3±4.4</td>
</tr>
<tr>
<td>Ang II+17-β-E, 10 nmol/L</td>
<td>117.8±12.5</td>
</tr>
<tr>
<td>Ang II+17-β-E, 100 nmol/L</td>
<td>107.2±10.8</td>
</tr>
<tr>
<td>Ang II+17-β-E, 1000 nmol/L</td>
<td>122.3±11.6</td>
</tr>
<tr>
<td>Ang II+17-β-E+Tam</td>
<td>116.9±16.2</td>
</tr>
<tr>
<td>Ang II+17-β-E+ICI</td>
<td>108.4±7.3</td>
</tr>
<tr>
<td>Ang II+17-α-E, 10 nmol/L</td>
<td>107.7±7.5</td>
</tr>
<tr>
<td>Ang II+17-β-E+Act-D</td>
<td>95.4±2.4</td>
</tr>
</tbody>
</table>

The top part of the table shows leukocyte rolling velocity in animals when Ang II superfusion was either continued or supplemented with 17-β-E or supplemented with 17-β-E in animals pretreated with tamoxifen (Tam) and ICI 182,780 (ICI) or supplemented with 17-α-E or 17-β-E+actinomycin D (Act-D) 30 minutes after Ang II superfusion. Leukocyte rolling velocity was measured 30 minutes later. All values are presented as mean±SEM. †P<0.05 or ‡P<0.01 relative to the control group (0 minutes).

The bottom part of the table shows leukocyte rolling velocity in animals pretreated with saline, Tam, or ICI 30 minutes before buffer superfusion. This parameter was measured in the following groups: saline, Tam, ICI, Tam+losartan, ICI+losartan, Tam+RMP-1, and Tam+WT-3. All values are presented as mean±SEM. †P<0.01 relative to the saline group at 30 minutes.

transillumination. The exposed mesentery was continuously superfused with warmed bicarbonate buffer saline (pH 7.4). An orthoscopic microscope (Nikon Optiphot-2, SMZ21) equipped with a ×20 objective lens (Nikon SLDW) and a ×10 eyepiece allowed tissue visualization. A video camera (Sony SSC-C350P) mounted on the microscope projected images onto a color monitor (Sony Trinitron PVM-14N2E), and these images were captured on a videotape (Sony RMP-1, and Tam). The bottom part of the table shows leukocyte rolling velocity in animals pretreated with tamoxifen (Tam) and ICI 182,780 (ICI) or supplemented with 17-β-E, 100 nmol/L 10.8, 62.9±6.9†, 94.9±12.9, 83.3±10.0, 42.6±6.8‡, 49.7±7.4‡, 48.3±2.1†, 92.8±2.8, 101.1±7.4.

Experimental Protocol

All preparations were left to stabilize for 30 minutes, and baseline (time 0) measurements of leukocyte rolling flux and velocity, leukocyte adhesion, leukocyte emigration, mean arterial blood pressure (MABP), Vrbc, shear rate, and venular diameter were obtained. The superfusion buffer was then supplemented with 1 nmol/L Ang II (Sigma). Recordings were performed for 5 minutes at 15-minute intervals over a 60-minute period, and the aforementioned leukocyte and hemodynamic parameters were measured. After 30 minutes of Ang II superfusion, and once its inflammatory effects were patent, 17-β-E (Sigma, 1 to 1000 nmol/L) was added to the superfusate and recordings were performed for 5 minutes at 15-minute intervals over an additional 30-minute period. On the basis of these initial experiments, 10 nmol/L 17-β-E was used for the remainder of the experiments. To investigate the preventive effect of 17-β-E, in another group of animals, 17-β-E 10 nmol/L was co-superfused with Ang II for 60 minutes at the beginning of the experiment. In another set of experiments, animals were pretreated with 17-β-E receptor antagonists tamoxifen (Sigma, 0.6 mg/kg IP) or ICI 182.780 (Zeneca Pharmaceuticals, 5 mg/kg IP) 30 minutes before Ang II superfusion. To determine the effect specificity of 17-β-E, the buffer was supplemented with 17-α-estradiol (17-α-E, Sigma, 10 nmol/L) after 30 minutes of Ang II superfusion. To elucidate if the effects exerted by 17-β-E are mediated at the level of gene transcription, actinomycin D (Act-D) (Sigma, 5 μmol/L) was co-superfused with the hormone for the same period of time.

To determine the potential origin of the nitric oxide (NO) released by 17-β-E, a selective inhibitor of neuronal NO synthase (nNOS), 7-nitroindazole (Sigma, 100 μmol/L), was coadministered with 17-β-E after 30 minutes of Ang II superfusion. The dose used was found to be selective for this enzyme isoform in a previous in vivo study. To investigate whether an estrogen blockade produces leukocyte–endothelial cell interactions, a first group of animals was pretreated with tamoxifen (0.6 mg/kg IP), a second group with ICI 182.780 (5 mg/kg IP), and a third group with saline 30 minutes before buffer superfusion. To investigate if Ang II is involved in the leukocyte recruitment provoked by a lack of estrogens, two groups of animals were pretreated with an AT1 Ang II receptor antagonist (losartan, 10 mg/kg IV) 10 minutes before antagonist administration. Losartan was kindly donated by Merck, Sharp & Dohme, Madrid, Spain. Finally, to elucidate which adhesion molecules are involved in these responses, three groups of animals were pretreated with tamoxifen. The first received only tamoxifen, the second was pretreated with a function blocking anti-rat P-selectin mAb (RMP-1, 2.5 mg/kg IV), and the third with a function blocking mAb against CD18-integrins.
Antibodies were administered 15 minutes before tamoxifen injection. Antibodies against rat-P-selectin (RMP-1) and rat-CD18 (WT-3) were acquired as previously stated.\textsuperscript{17,18}

**Cell Culture**

Human umbilical vein endothelial cells (HUVECs) were isolated by collagenase treatment\textsuperscript{19} and maintained in human endothelial cell–specific medium EBM-2 (Clonetics) supplemented with EGM-2 (Clonetics). FBS was deprived of estrogen by charcoal stripping. HUVECs were grown to confluence and used up to passage 2 for the experiment. HUVECs were grown on 24-well culture plates and were serum-starved for 8 hours before every experiment in phenol red–free medium 199 (Gibco) containing no FBS.

**Prostacyclin Assay**

Cells were incubated with 17-\textbeta-E 0.1 to 1000 nmol/L for 15 and 30 minutes, ICI 182,780 (10 \textmu mol/L) was added to the wells 30 minutes before 17-\textbeta-E (10 nmol/L) stimulation. At the end of the experiment, the supernatants were collected and the amount of prostacyclin, calculated as the concentration of the stable hydrolysis product, 6-keto-prostaglandin F\textsubscript{1a}, was assessed in triplicate using a commercial EIA kit (Cayman Chemical Company).

**Statistical Analysis**

All values are presented as mean±SEM. Data within groups were compared using one-way ANOVA with a Newman-Keuls post hoc correction for multiple comparisons. Statistical significance was set at \(P<0.05\).
Results

Ang II 1-nmol/L superfusion promoted a significant increase in leukocyte rolling flux (82.0±10.7 versus 20.8±1.4 cells/min), leukocyte adhesion (10.3±1.9 versus 0.8±0.3 cells/100 μm), and leukocyte emigration (2.8±0.6 versus 0.3±0.3 cells/field) at 60 minutes compared with basal values. These results are consistent with previous studies from our laboratory. After 30 minutes of superfusion, Ang II caused significant increases in all leukocyte parameters versus values detected at time 0 (Figure 1). When 17-β-E (10 nmol/L) was administered after 30 minutes of Ang II superfusion, leukocyte responses were reduced by 55.1%, 72.7%, and 70.9%, respectively, 30 minutes later (Figure 1). This dose caused maximal and most consistent effect and was therefore used for the remainder of the experiments. The decrease in leukocyte rolling velocity induced by Ang II was reversed by cosuperfusion with 10 to 1000 nmol/L of 17-β-E (Table). Concomitant superfusion of Ang II and 17-β-E from the beginning of the experiment resulted in a complete inhibition of Ang II–induced leukocyte–endothelial cell interactions, inhibiting leukocyte rolling flux, adhesion, and emigration by 95.1%, 86.8%, and 90.0%, respectively, after 60 minutes of superfusion. These responses were receptor-mediated, because pretreatment of the animals with the estrogen receptor antagonists, tamoxifen, or ICI 182,780 reversed the antiinflammatory activity of 17-β-E (Figure 2 and Table). In addition, these effects were estrogen-specific, because superfusion with the same dose of 17-α-E had no effect on Ang II–induced leukocyte responses (Figure 3 and Table). Furthermore, cosuperfusion of Act-D with 17-β-E did not modify the inhibitory effects exerted by the hormone. Hemo-

Figure 3. Effect of 17-α-E on Ang II–induced leukocyte rolling flux (A), leukocyte adhesion (B), and leukocyte emigration (C) and effect of Act-D on 17-β-E inhibitory responses to Ang II–induced leukocyte rolling flux (A), leukocyte adhesion (B), and leukocyte emigration in rat mesenteric postcapillary venules (C). The mesentery was superfused with Ang II (1 nmol/L), and after 30 minutes of superfusion, 17-β-E (10 nmol/L), 17-α-E (10 nmol/L), or 17-β-E (10 nmol/L) plus Act-D (5 μmol/L) was added to the superfusate. Animals were divided into 4 groups. Ang II superfusion was either continued (n=5) or supplemented with 17-β-E (n=5), 17-α-E (n=5), or 17-β-E+Act-D (n=4). Parameters were measured 15 and 30 minutes later. Results are presented as mean±SEM. *P<0.05 or **P<0.01 relative to the control value (0 minutes) in the untreated group; +P<0.05 or ++P<0.01 relative to the untreated group (Ang II+buffer).
dynamic parameters such as MABP and shear rates remained the same throughout the experimental period in both the untreated group and the groups subjected to varying treatments (data not shown).

Because estrogens can provoke the release of NO and prostacyclin from the vascular wall and both vasodilators have antiadhesive properties, this possibility was additionally investigated. When 17\(\beta\)-E was cosuperfused after 30 minutes of suffusion with a NOS or a cyclooxygenase (COX) inhibitor, leukocyte responses were not modified, indicating a clear role for both vasodilators in estrogen-elicited antiadhesive properties (Figure 4). Additionally, inhibitory responses induced by 17\(\beta\)-E on Ang II–induced leukocyte–endothelial cell interactions were not affected by nNOS inhibition with 7-nitroindazole. Indeed, leukocyte rolling flux (34.2±2.7 cells/min), leukocyte adhesion (2.8±0.6 cells/100 \(\mu\)m venule), and leukocyte emigration (1.8±0.4 cells/field) after 60 minutes of superfusion with Ang II+17\(\beta\)-E+7 nitroindazole did not differ from values detected in the Ang II+17\(\beta\)-E–treated group at the same time point (34.6±6.8 cells/min, 3.4±0.4 cells/100 \(\mu\)m venule, and 1.0±0.4 cells/field, respectively). Thus, these results indicate that NO released by estrogens has an endothelial origin. In addition, to demonstrate the involvement of prostacyclin in the rapid responses elicited by estrogens, HUVECs were incubated with 17\(\beta\)-E at different concentrations for 15 or 30 minutes. 17\(\beta\)-E caused a dose-dependent release of prostacyclin (Figure 5), which was maximal after 30 minutes of incubation with 10 nmol/L (1.15±0.09 versus 0.48±0.04 ng prostacyclin/mg of cellular protein). This result is consistent with the effects achieved in vivo.
Furthermore, tamoxifen administration induced a significant increase in leukocyte rolling flux (87.0 ± 15.1 versus 27.7 ± 8.1 cells/min), leukocyte adhesion (8.5 ± 1.8 versus 1.7 ± 0.9 cells/100 μm) and leukocyte emigration (2.5 ± 0.5 versus 0.7 ± 0.3 cells/field) 90 minutes after its administration compared with values detected in the saline-treated group for the same time period. Similar results were obtained when ICI 182,780 was intraperitoneally administered. Interestingly, leukocyte responses after 90 minutes of exposure to tamoxifen were inhibited through systemic administration of losartan by 85.4%, 74.4%, and 61.5%, respectively, after an anti–CD18 mAb (WT-3) administration of tamoxifen were significantly reduced by 100%, 81.4%, and 61.5%, respectively (Figure 6), and by 46.0%, 67.7%, and 53.3%, respectively, in animals intraperitoneally injected with ICI 182,780 (Figure 6). In addition, increases in leukocyte rolling flux, adhesion, and emigration after 90-minute administration of tamoxifen were significantly reduced by an anti–P-selectin mAb (RMP-1) pretreatment by 100%, 81.4%, and 61.5%, respectively, and by 21.6%, 81.4%, and 61.5%, respectively, after an anti–CD18 mAb (WT-3) preadministration (Figure 7). Moreover, tamoxifen and ICI 182,780 provoked a significant decrease in leukocyte rolling velocity after 90-minute administration; however, this parameter was maintained at basal levels when animals received the aforementioned pretreatments (Table). Again, MABP and shear rate 90 minutes after saline, tamoxifen, or ICI 182,780 administration in animals untreated and pretreated with losartan, RMP-1, or WT-3 were not altered at any time during the experimental period (data not shown).

Discussion

In the present study, we demonstrated that estrogens not only diminish but also prevent Ang II–induced leukocyte–endothelial cell interactions in vivo. This effect is receptor-mediated, because tamoxifen or ICI 182,780 pretreatment resulted in the total abolition of estrogen-induced responses. Indeed, estrogens seem to attenuate vasoconstrictive responses to multiple agonists, including Ang II,20 and it is therefore likely that leukocyte–endothelial cell interactions induced by this peptide hormone are also affected. Although Ang II–induced leukocyte recruitment is mediated through the release of reactive oxygen species21 and antioxidant properties have been attributed to 17-β-E because of the presence of a phenolic group in its structure, the lack of effect of 17-α-E when it was cosuperfused with this peptide hormone suggests that 17-β-E acts through a different mechanism. The fast rate of the response observed indicates that the process does not require the classical nuclear effects of the hormone. In fact, cosuperfusion with the transcriptional inhibitor Act-D did not modify the inhibitory responses elicited by 17-β-E. This observation is consistent with a newly discovered, nongenomic physiological role for estrogen receptors in endothelial cells. In this context, it has recently been shown that estrogen receptors are found in both the endothelial cell surface and the leukocyte surface and that they exert different effects through interaction with their α-subtype receptor.22,23

It is also interesting to note that in the present study, the protective effect exerted by estrogens is dependent on the dose used. In fact, inhibitory effects of 17-β-E were observed at 10 and 100 nmol/L but not at a dose <1 nmol/L. Higher doses of 17-β-E, such as 1 μmol/L, provoked a minor reduction in the leukocyte–endothelial cell interactions induced by Ang II. Although physiological doses of estrogens are close to 1 nmol/L, in our study, maximum effects of 17-β-E were detected at 10 nmol/L. Because this was the dose present in the superfusion buffer, it is possible that the effects observed were attributable to a much lower dose, because 17-β-E had to reach the intravascular space after passing various biological barriers. Nevertheless, a similar dose was found to be effective in other studies of the effects of estrogens,22,24 and this level of estrogens can be achieved during pregnancy.25

Our results highlight that the effects of estrogens are mediated through a direct action on the endothelium and via NO and prostacyclin release. In this way, 17-β-E did not provoke any changes in the increased leukocyte–endothelial cell interactions detected after acute NOS or COX inhibition. The results of our study are in contrast to our expectations. We expected that prostacyclin release by 17-β-E would attenuate L-NAME–induced leukocyte–endothelial cell inter-
actions and that quick production of NO would exert a similar effect in the leukocyte recruitment caused by indomethacin superfusion. In fact, surprisingly, both enzyme inhibitors provoked similar responses; therefore, it is possible that acute NOS inhibition results in acute COX inhibition and vice versa, as was noted in articular cartilage. Furthermore, we can deduce that the NO released by these hormones has an endothelial origin, because the selective nNOS inhibitor 7-nitroindazole did not modify the estrogens’ diminution of Ang II–induced responses. There is evidence that estrogens provoke rapid NOS activation independently of gene transcription without inducing upregulation of NOS. In the present study, we have revealed a similar pattern of prostacyclin production triggered on estrogen stimulation. Indeed, significant increases in these prostaglandin levels were detected after 15-minute incubation of HUVECs with 17-β-E. These results are consistent with those obtained in a previous study, in which we demonstrated the strong inhibitory responses elicited by iloprost, a prostacyclin analogue, on Ang II–induced leukocyte–endothelial cell in-
Interactions in vivo. Thus, the rapid prostacyclin and NO release from the endothelium may account for the quick antiinflammatory activity exerted by estrogens in the present study.

Inhibition of leukocyte recruitment by estrogens has been demonstrated in several in vivo studies. Despite these findings, the adhesive mechanisms involved in these leukocyte responses are not precisely known. Although some authors have found that estrogen administration can reduce intercellular adhesion molecule-1 expression, others have demonstrated the downregulation of vascular cell adhesion molecule-1 expression, decreased E-selectin expression, or both. However, the rapid responses elicited by estrogens indicate that downregulation of adhesion molecules other than intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin is involved in the inhibitory responses observed in the present study. In this context, one possible candidate is P-selectin. Indeed, we have recently demonstrated that Ang II–induced leukocyte–endothelial cell interactions in vivo occur via endothelial P-selectin upregulation. In addition, lower plasma levels of P-selectin have been detected in premenopausal women compared with those encountered in men, and the administration of a single dose of estradiol significantly decreases the plasma levels of this adhesion molecule in men.

Finally, to additionally investigate the consequences of estrogen deficiency occurring in men and postmenopausal women, we mimicked said condition by systemic treatment
with the estrogen receptor antagonists tamoxifen or ICI 182,780. It is worthy to note that serum levels of 17-β-E in male adult rats range between 40 and 110 pmol/L.53 In the male rat, most of this hormone is not testicular in origin but rather is derived from the adrenal glands.54 These treatments provoked a significant increase in leukocyte–endothelial cell interactions 90 minutes after administration, which were abolished by losartan pretreatment. Thus, leukocyte responses elicited by lack of estrogens are mediated through the interaction of Ang II with its AT1 receptor subtype. In accordance with these findings, we have recently demonstrated that COX inhibition–induced and NOS inhibition–induced leukocyte–endothelial cell interactions are attributable to the deleterious action of Ang II.55 In addition, leukocyte–endothelial cell interactions elicited by estrogen receptor blockade were P-selectin–dependent and CD18-integrin–dependent, because administration of mAbs, which blocked the function of these adhesion molecules, dramatically reduced tamoxifen-induced leukocyte responses. These results suggest that a lack of estrogens results in a deficiency of endothelial-derived vasodilators such as prostacyclin and NO. In fact, L-NAME–induced leukocyte–endothelial cell interactions are P-selectin–dependent and CD18-integrin–dependent.36,37 and COX inhibition causes significant vascular P-selectin and CD18-integrin expression within 1 hour.38 Considered as a whole, these findings suggest that low levels of estrogens, which can be encountered in men and postmenopausal women, provoke endothelial barrier dysfunction owing to lack of NO and prostacyclin. The absence of these vasodilators provokes the exposure of the endothelium to Ang II and the upregulation of adhesion molecules such as P-selectin and CD18-integrins, which results in the leukocyte recruitment and the subsequent subendothelial leukocyte infiltration associated with the onset of the atherogenic lesion.

In conclusion, we have demonstrated in the present study that estrogen antiatherogenic activity can be Explained, in part, by the inhibition of the inflammatory response induced by Ang II. This effect is primarily mediated by estrogen interaction with its α-receptor subtype, which is present in the endothelial cell surface, and the subsequent immediate activation of eNOS and COX. The results obtained indicate that the increased risk of cardiovascular disorders in men and postmenopausal women can be attributed to a lack of estrogens, which causes a disruption of vascular balance and a deficiency in endothelial vasodilators. In this way, the endothelium is exposed to the deleterious action of Ang II, provoking the subendothelial infiltration of the adhered leukocytes, which could constitute a mechanism in the onset of the atherosclerotic lesion caused by an estrogen deficiency.

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