Diabetes Undermines Estrogen Control of Inducible Nitric Oxide Synthase Function in Rat Aortic Smooth Muscle Cells Through Overexpression of Estrogen Receptor-β

Adriana Maggi, PhD*; Andrea Cignarella, PhD*; Alessia Brusadelli, PhD; Chiara Bolego, PhD; Christian Pinna, PhD; Lina Puglisi, PhD

Background—Previous reports from our group have shown that 17β-estradiol reduces the synthesis and activity of inducible nitric oxide synthase (iNOS) in rat aortic smooth muscle cells (SMC) in response to inflammatory mediators. In this study, we investigated the effect of 17β-estradiol on iNOS function in aortic SMC from streptozotocin-diabetic rats.

Methods and Results—Comparative analysis of NO release and of iNOS mRNA and protein content after 24-hour stimulation with a cytokine mixture revealed milder iNOS activation in diabetic than in control SMC. Furthermore, 17β-estradiol dose-dependently blocked iNOS synthesis and activity in control but not in diabetic SMC. The defective estrogen response in diabetic SMC at 24 hours could not be attributed to reduced expression of estrogen receptors (ER). In fact, mRNA and protein levels of ERα and, to a greater extent, of ERβ, were increased in diabetic compared with nondiabetic SMC. Cytokines decreased ERα and ERβ expression in both groups. However, 17β-estradiol dose-dependently restored the expression of ERα but further downregulated that of ERβ, indicating a differential regulation of ER isoforms.

Conclusions—Estrogenic control of iNOS was impaired in diabetic SMC. This was associated with a larger increase of ERβ than of ERα protein, whereas 17β-estradiol regulated the two isoforms in an opposite fashion. Thus, modifications in the estrogen modulation of iNOS and in the expression pattern of ER may be involved in diabetic vascular dysfunction. (Circulation. 2003;108:211-217.)

Key Words: myocytes • nitric oxide synthase • diabetes mellitus • hormones

Diabetes is a cardiovascular disease.1 Substantial evidence indicates that diabetic vascular dysfunction is associated with marked alterations of nitric oxide (NO) pathways.2 In the presence of high blood glucose levels, the increased vascular generation of oxygen-derived free radicals, primarily superoxide anions, quenches the biological activity of NO.3-4 This may result in a transient increase in NO synthesis through upregulation of endothelial NO synthase (eNOS) in endothelial cells, and even more so through activation of the inducible NOS (iNOS) isoform in smooth muscle cells (SMC) and macrophages. The sustained accumulation of high NO levels generated by iNOS can be toxic through reaction with superoxide to yield peroxynitrite, thereby playing a central role in the pathophysiology of inflammation and oxidant stress.5 Thus, the iNOS-mediated increase in NO formation may be a major mediator of diabetic vascular dysfunction. Accordingly, functional expression of iNOS has been reported in SMC from the superior mesenteric arteries of rats with chronic diabetes.6

We previously showed that diabetes impairs vascular function in female streptozotocin-diabetic rats by interfering with NO pathways.7 In women, diabetes abolishes the gender advantage in cardiovascular risk typical of premenopause.8 This protection is thought to be mediated, at least in part, by endogenous 17β-estradiol (E2) through mechanisms still under investigation.

Thus far, two estrogen receptors (ER) have been identified: ERα and ERβ. These proteins are encoded by two separate genes, are distinct structurally and functionally, and are expressed in endothelial cells,9 SMC,10,11 and macrophages.12 In endothelial cells, E2 increases the expression of eNOS, thereby controlling the tone of underlying SMC,13 and may also rapidly activate eNOS through ERα-mediated mechanisms not involving gene expression.14,15 Conversely, E2 blocks the synthesis of iNOS induced by inflammatory stimuli in SMC11 and macrophages,12 suggesting that the beneficial effect of the hormone arises from a combined action on different vascular cell types.
The aim of the present study was to further investigate the effects of E2 on the expression and activity of iNOS in cultured aortic SMC from diabetic rats. In this study, we show that iNOS response to inflammatory events in SMC from diabetic rats is less sensitive to estrogen, probably on account of altered ERα/ERβ ratio. These findings indicate possible mechanisms underlying the increased risk of cardiovascular disease in diabetic premenopausal women.

Methods

Materials

E2, L-arginine, sepiapterin, ascorbic acid, L-nitroarginine-methyl ester (L-NAME), 5-ethylisothiourea (EIT), and streptozotocin (STZ) were obtained from Sigma. The anti-rabbit iNOS polyclonal antibody was purchased from Transduction Laboratories (Lexington, Ky). The anti-ERα and anti-ERβ antibodies were kindly provided by Dr Geoffrey L. Greene (University of Chicago). Oligodeoxynucleotides were synthesized by Amersham Biosciences.

Diabetes Induction

Diabetes was induced by intravenous injection of STZ (65 mg/kg) in male Sprague-Dawley rats weighing 200 to 225 g (Charles River, Calco, Italy). STZ was freshly dissolved in citrate buffer (pH 4.5); control animals were injected with vehicle. Diabetes induction was considered successful when glycosuria was detectable 1 week after treatment. When the animals were killed, they had plasma glucose levels >25 mmol/L. The procedures followed were in accordance with institutional guidelines of the University of Milan.

Cell Culture

SMC were obtained from aortic intimal-medial layers of nondiabetic and 28-day diabetic rats according to Ross.16 Cells were grown in medium 199 (M199) as previously described.11 The medium was replaced with phenol red–free M199 with 10% FCS for 5 days whenever the effects of E2 were tested. Twenty-four hours before replacing the medium, cells were synchronized in medium containing 25 mmol/L. The procedures followed were in accordance with institutional guidelines of the University of Milan.

Gene Expression Analysis

Total RNA was extracted from SMC (2×10^5 cells/well) and rat uterus (as a positive control for ER mRNA expression) with the Bio/RNA-X Cell kit (Bio/Gene, Kimbolton, UK). RNA was reverse transcribed using MMLV reverse transcriptase H(+) (Promega). Amplification of ERα and ERβ cDNAs was carried out with the use of published primer sequences.11 Amplification of iNOS cDNA was achieved by using the primer pair 5¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬¬...
substrate L-arginine or cofactor sepiapterin did not augment nitrite accumulation in the medium of diabetic SMC, suggesting that those were not limiting steps for iNOS activity. Similarly, addition of either ascorbic acid or lercanidipine (a vascular-selective calcium channel blocker with marked antioxidant properties) failed to affect nitrite production, suggesting that no increased oxidant burden impaired iNOS activity.

In both SMC groups, the cytokine-induced nitrite increase was reversed by the nonselective NOS inhibitor L-NAME and the relatively selective iNOS inhibitor EIT (Figure 2), suggesting that it was secondary to iNOS induction. After 24-hour cytokine challenge, nitrite levels were again greater in the media of control SMC than in those of diabetic SMC. However, at 48 hours, nitrite content in the two groups was comparable. Further investigation showed that such differences reflected a distinct temporal pattern of iNOS protein induction. In fact, we consistently measured the highest iNOS protein levels after 48-hour cytokine treatment in diabetic SMC and after 24 hours in control SMC (Figure 3). After 72 hours, iNOS protein levels decreased, yet were significantly higher than in untreated cells. This delayed response to cytokine stimulation in diabetes was not restricted to isolated SMC but was also observed in whole aortic rings in culture under identical conditions (not shown).

E2 Exerts Opposite Modulation of iNOS Synthesis in Control and Diabetic SMC

We then evaluated the content of iNOS mRNA and protein after E2 treatment. As expected, iNOS mRNA was undetectable in untreated SMC from either group, as shown by semiquantitative RT-PCR. In control SMC, cytokine stimulation for 24 hours increased iNOS mRNA content; a modest induction was observed in diabetic SMC as well. However, the overall effect of E2 was opposite in the two groups. In fact, E2 (10^{-11} to 10^{-9} mol/L) dose-dependently reduced iNOS mRNA levels in control SMC but increased iNOS mRNA in diabetic SMC severalfold compared with cytokine-treated diabetic and ∼2-fold compared with cytokine-treated control SMC (data not shown).

Western blot experiments showed that E2 reduced in a dose-dependent manner iNOS protein content in cytokine-treated control SMC (Figure 4A). By contrast, treatment with E2 did not affect iNOS protein levels in diabetic SMC despite increased mRNA levels. As shown in Figure 4B, after 48-hour cytokine stimulation, E2 was about as effective in control as in diabetic SMC at reducing iNOS protein content, indicating that the early difference in response to E2 between control and diabetic SMC leveled off at later time points. Similar results were obtained for iNOS activity (data not shown).

<table>
<thead>
<tr>
<th>Treatment/Concentration, ( \mu \text{mol/L} )</th>
<th>Nitrite, ( \mu \text{mol/mg protein} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>( 0.041 \pm 0.014 )</td>
</tr>
<tr>
<td>Cytokines</td>
<td>( 0.085 \pm 0.011^* )</td>
</tr>
<tr>
<td>Cytokines + L-arginine</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>( 0.067 \pm 0.009 )</td>
</tr>
<tr>
<td>100</td>
<td>( 0.068 \pm 0.017 )</td>
</tr>
<tr>
<td>500</td>
<td>( 0.073 \pm 0.010 )</td>
</tr>
<tr>
<td>Cytokines + ascorbic acid</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>( 0.077 \pm 0.013 )</td>
</tr>
<tr>
<td>500</td>
<td>( 0.064 \pm 0.016 )</td>
</tr>
<tr>
<td>2000</td>
<td>( 0.079 \pm 0.008 )</td>
</tr>
<tr>
<td>Cytokines + lercanidipine</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>( 0.068 \pm 0.011 )</td>
</tr>
<tr>
<td>1</td>
<td>( 0.089 \pm 0.012 )</td>
</tr>
<tr>
<td>5</td>
<td>( 0.084 \pm 0.015 )</td>
</tr>
<tr>
<td>Cytokines + sepiapterin</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>( 0.067 \pm 0.010 )</td>
</tr>
<tr>
<td>100</td>
<td>( 0.061 \pm 0.015 )</td>
</tr>
<tr>
<td>300</td>
<td>( 0.073 \pm 0.006 )</td>
</tr>
</tbody>
</table>

SMC were incubated with cytokines for 24 hours in the presence of increasing concentrations of the indicated compounds. At the end of incubation, medium aliquots were assayed for nitrite content by the Griess’ reaction (see Methods section). Data are expressed as mean±SEM of 3 to 6 independent experiments in duplicate. ^*P<0.05 vs control.

Figure 2. Nitrite production by control and diabetic SMC after 24-hour and 48-hour incubation with cytokines. SMC were grown in M199 medium+10% FCS until confluence, synchronized in 0.4% FCS for 24 hours, and stimulated as described in the legend to Figure 1 for the indicated time in the presence of the NOS inhibitors L-NAME (0.3 mmol/L) and EIT (10 \( \mu \text{mol/L} \)). Nitrite production was measured in medium aliquots by Griess’ reaction. Data are expressed as mean±SEM of 5 to 7 independent experiments, each performed in duplicate. ^*P<0.01 vs basal; §P<0.01 vs cytokines.
Expression Correlates With Decreased ER

diabetic with respect to nondiabetic SMC, the levels of ER
dependently restored ER
and this was dose-dependently reversed by E2. Experimental
dependently reduced ER
untreated diabetic and nondiabetic SMC and (2) both ER
The antithetical control of E2 on iNOS expression in SMC
In this study we show that the cytokine-mediated iNOS
protein level, which was
increased by 40% to 50% only (Figure 6). In
incubation could not be ascribed to deficiencies in iNOS
from diabetic and nondiabetic rats after 24-hour cytokine
treatment, ER
mRNA decreased by
50% in
11to 10
9mol/L) dose-
and ER
5 and 6) provides further evidence for the potential divergent
effects of the two receptors.28,30 By upregulating ER
and ER
expression in both groups. On
cytokine treatment, ER
mRNA decreased by ∼50% in
diabetic SMC and fell to barely detectable levels in control
SMC (Figure 5). In sharp contrast to ER
, E2 dose-
dependently restored ER
mRNA in diabetic SMC but had no
detectable effect in control SMC.

In contrast to the strong accumulation of ERα mRNA in
diabetic with respect to nondiabetic SMC, the levels of ERα protein were increased by 40% to 50% only (Figure 6). In
both SMC groups, cytokines strongly decreased ERα levels, and this was dose-dependently reversed by E2. Experimental
diabetes, on the other hand, had a marked impact on ERβ protein level, which was >than 2-fold higher than in nondiabetic SMC. ERβ content was not affected by cytokine
treatment but was significantly diminished by E2, particularly
in diabetic SMC (Figure 6).

Discussion

In this study we show that the cytokine-mediated iNOS
activation and the estrogen response were very different in
aortic SMC from diabetic compared with control rats and that
this effect was associated with a significant change in ERα and ERβ relative content. These differences may be relevant
to diabetic vascular dysfunction.

Data from our group and other groups indicate that the E2
antagonism to the synthesis of inflammatory proteins is
mediated by ER because it occurs in cell cultures at hormone
concentrations compatible with ER transcriptional activation,
is blocked by specific ER antagonists, and is linked to ER
expression.11,12,23,24 In vitro transfection experiments show
that ER is indispensable to E2 regulation of iNOS transcription.
There is little evidence, however, for a direct interaction of estrogen with the iNOS promoter,25 which lacks canonical
estrogen responsive elements. As proposed for the effect of
estrogens on other molecules of the inflammatory cascade, it
is conceivable that iNOS mRNA synthesis is controlled by
ERs by interaction with transcription factors such as NF-κB,
AP-1, or STATs.26,27 Paech et al28 reported that E2 has
opposite influence on NF-κB and AP-1 transcription activity,
depending on its association with ERα or ERβ. Considering
a differential effect of the two ER subtypes on iNOS
expression, we propose that the altered ERα/ERβ ratio
reported here for diabetic SMC, and in particular the remark-
able upregulation of ERβ, underlies the loss of E2 negative
control on iNOS activity. ERα and ERβ expression was
increased in diabetic compared with control SMC, with a
relative increase in ERα and ERβ protein levels of ∼1.4- and
2.5-fold, respectively. Although no experiments were per-
formed in this study to distinguish functionally between ERα
and ERβ, our findings suggest that the increased ERβ
expression was associated with the defective response to E2
and to cytokines observed in diabetes. Supporting this hy-
thesis are recent studies in ERβ-deficient mice describing
an important role for ERβ in regulating vascular function and
blood pressure.29 The authors showed that ERβ is involved
in estrogen-induced accumulation of iNOS protein in endothel-
ium-denuded aortic rings as well as iNOS gene transcription
in normal vessels. This lends further support to the view that
the altered estrogenic control of iNOS function in diabetic
SMC was mediated mainly by ERβ.

The observation that ERα and ERβ protein content was
regulated by E2 in an opposite fashion in aortic SMC (Figures
5 and 6) provides further evidence for the potential divergent
effects of the two receptors.28,30 By upregulating ERα, E2 may
enhance the protective antiinflammatory effects mediated by
this receptor subtype in these cells while limiting potential
proinflammatory effects with ERβ downregulation. Interest-
ingly, enhanced transcription of both ER subtypes occurred in
diabetic cells, although the increase in protein level was more
evident for ERβ. It is conceivable that ER protein underwent
translational regulation, as was the case for iNOS; this
phenomenon has been reported to occur in diabetes.31,32
Accordingly, the cytokine-dependent synthesis of iNOS was
delayed and significantly blunted in diabetic cells possibly
due to altered levels of transcription factors or of intracellular
signaling molecules such as intracellular Ca2+, as suggested
by previous studies in SMC.33 The cytokine response itself
was blunted but not abolished in diabetic SMC at the 24-hour
time point. Therefore, the altered time course of iNOS
response to cytokines and the corresponding loss of estro-

Figure 3. Time course of iNOS protein synthesis in control and
diabetic SMC. SMC were grown and stimulated as described in
the legend to Figure 2. Amount of iNOS in cell lysates was
quantified by scanning densitometry. Representative Western
blot of iNOS is shown. Data are expressed as mean±SEM of 3
independent experiments. *P<0.01 vs control.

Loss of E2-Inhibitory Control on iNOS Gene
Expression Correlates With Decreased ERα and
Increased ERβ Expression

The antithetical control of E2 on iNOS expression in SMC
from diabetic and nondiabetic rats after 24-hour cytokine
incubation could not be ascribed to deficiencies in iNOS
system. This led us to investigate whether diabetes had
altered the expression pattern of ERs. It was apparent that (1)
the mRNA of ERα was more abundant than that of ERβ in
untreated diabetic and nondiabetic SMC and (2) both ERα
and ERβ mRNA levels were consistently greater in diabetic
than in control SMC under any condition tested (Figure 5).
Cytokine incubation for 24 hours markedly reduced ERα
mRNA in diabetic SMC but had no
expression in both groups. On
cytokine treatment, ERβ mRNA decreased by ∼50% in
diabetic SMC and fell to barely detectable levels in control
SMC (Figure 5). In sharp contrast to ERα, E2 dose-
dependently restored ERβ mRNA in diabetic SMC but had no
detectable effect in control SMC.

In contrast to the strong accumulation of ERα mRNA in
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treatment but was significantly diminished by E2, particularly
in diabetic SMC (Figure 6).
genic effect on this phenomenon, along with the changes in the ER expression pattern, appeared to be part of diabetic vascular dysfunction in aortic SMC.

Activation of iNOS in SMC leads to rapid production of high amounts of NO, which causes pathological vasodilation. In addition, excess NO may interact with superoxide to generate peroxynitrite, which in turn modifies the function of several proteins, contributing to the tissue damage occurring in vascular sepsis and inflammation.\textsuperscript{18} Our results may partially explain why diabetes abolishes the gender-specific...
vascular protection afforded by estrogen reported in epidemiological studies. Thus, understanding the mechanism for the vascular alterations in estrogen response and ER levels in diabetes would be particularly relevant from a clinical perspective. Estrogen inhibits the iNOS activation associated with ovariectomy, uraemia, transplantation, and inflammatory stimuli. Estrogen also appears to be effective in clinical conditions sharing inflammatory features such as multiple sclerosis, Alzheimer’s disease, osteoporosis, and periodontitis, indicating that the beneficial effects of estrogen reflect its anti-inflammatory action. In view of the possible role of ERβ in the development of diabetic vascular dysfunction, it would be interesting to assess the role of ERβ in other inflammatory disorders in which the hormone exerts protective effects. These studies might eventually lead to the development of novel drugs for prevention and treatment of inflammatory disease affecting the aging woman.

Acknowledgments

This work was supported by grants from the Italian Association for Cancer Research (A.M.), Ministry of Education and University (II National Research Program on Drugs and COFIN), European Union (QLRT-2001-02221), and CARIPLO Foundation (A.M. and A.C.).

References

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Circulation. 2003;108:211-217; originally published online June 23, 2003;
doi: 10.1161/01.CIR.0000079311.39939.94

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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