Genistein restores caveolin-1 and AT-1 receptor expression and vascular function in large vessels of ovariectomized hypertensive rats

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Abstract

Objective: The soy-derived phytoestrogen genistein improves endothelial function in postmenopausal women and ovariectomized (OVX) normotensive rats. We hypothesized that genistein would improve vascular reactivity involving changes in endothelial nitric oxide synthase (NOS) expression and its regulatory proteins (caveolin and calmodulin), angiotensin II receptor, and/or oxidative status in OVX spontaneously hypertensive rats (SHRs).

Design: After ovariectomy or sham operation, 23-week-old female SHRs received either 17β-estradiol (2 mg/kg/wk SC), genistein (10 mg/kg/d by gavage), or placebo.

Results: In OVX rats, final body weight was increased and uterus weight was decreased, and these values were reduced and increased, respectively, by 17β-estradiol but unaffected by genistein. Acetylcholine-induced endothelium-dependent vasorelaxation was significantly blunted in aortas from OVX placebo SHRs. The contractions induced by the NOS inhibitor L-NAME and angiotensin II in OVX placebo were lower and higher, respectively, than in sham rats. Estradiol and genistein restored these functional changes. Aortic endothelial NOS and calmodulin-1 expression were unchanged, whereas caveolin-1 and angiotensin II receptor expression was increased in OVX rats. Estradiol and genistein treatment did not modify endothelial NOS, but normalized caveolin-1 and angiotensin II receptor and increased calmodulin-1 expression. Vascular superoxide production was increased in OVX placebo and normalized by both estradiol and genistein.

Conclusions: Genistein prevented all cardiovascular changes induced by estrogen depletion in SHRs to a similar extent as estradiol but had no uterotrophic effect. The present findings may help to explain the potential benefits of genistein as a therapeutic agent for preventing menopausal vascular complications, especially in hypertensive women.

protein, are believed to mediate the beneficial cardiovascular effects of soy consumption.\textsuperscript{17,18} Genistein, the prototypical isoflavone, relaxes isolated rat arteries through NO-dependent\textsuperscript{19} and -independent\textsuperscript{20} mechanisms and enhances the dilator response to acetylcholine (ACh) in coronary arteries from atherosclerotic female macaques\textsuperscript{21} and in aortas from male spontaneously hypertensive rats (SHRs).\textsuperscript{11} In vivo, genistein has also been reported to improve endothelial function in ovariectomized (OVX) normotensive rats\textsuperscript{22} and in SHRs.\textsuperscript{23} In addition, genistein or soy supplements have been proven to be effective in protecting endothelial dysfunction in men and healthy postmenopausal women.\textsuperscript{24} Genistein binds to both ER\textsubscript{x} and ER\textsubscript{B}, but, in contrast to estradiol, it shows greater affinity for ER\textsubscript{B}.\textsuperscript{25} In addition to its estrogenic effects, it also shows antioxidant effects.\textsuperscript{26,27}

We have investigated whether genistein and 17\textbeta-estradiol exerted a similar in vivo protection against the decline in vascular function in an experimental model of endothelial dysfunction induced by both blood pressure increase and ovariectomy in female rats. This model reproduces the impairment in endothelial function observed in hypertensive postmenopausal women.\textsuperscript{26} Genistein binds to both ER\textsubscript{x} and ER\textsubscript{B}, but, in contrast to estradiol, it shows greater affinity for ER\textsubscript{B}.\textsuperscript{25} In addition to its estrogenic effects, it also shows antioxidant effects.\textsuperscript{26,27}

**METHODS**

**Animals and experimental groups**

Experiments followed our institutional guidelines for the ethical care of animals. Female SHR aged 23 weeks (Harlan Laboratories, Barcelona, Spain) were maintained (five per cage, 24 ± 1°C, 12-h dark/light cycle) on soy-free chow (AIN 76). SHRs are an inbred rat strain derived from Wistar rats, which are genetically predisposed to develop hypertension spontaneously, resembling human essential hypertension. Rats were OVX or sham-operated under anesthesia (2.5 mL/kg equithesin). Three weeks after surgery, animals were divided into the following groups (eight in each group): sham, OVX, OVX-genistein (10 mg/kg/d by gavage), and OVX-estradiol (2 mg/kg/wk, ie, equivalent to 0.28 mg/kg/d SC) and followed for 5 weeks. All animals were given placebo: 1 mL of 1% wt/vol methylcellulose daily by gavage (vehicle for genistein) and/or 0.5 mL sesame oil SC once per week (vehicle for estradiol).

**Blood pressure and plasma measurements**

Systolic blood pressure was measured weekly 18 to 20 hours after administration of the drugs in conscious, prewarmed, restrained rats by tail-cuff plethysmography.\textsuperscript{29} Plasma estrogens were measured by electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany) at the end of the treatment.

**Vascular reactivity studies**

Descending thoracic aortic rings (3 mm) were dissected and mounted in organ chambers filled with Krebs solution (composition in mmol/L: NaCl 118, KCl 4.75, NaHCO\textsubscript{3} 25, MgSO\textsubscript{4} 1.2, CaCl\textsubscript{2} 2, KH\textsubscript{2}PO\textsubscript{4} 1.2, and glucose 11) at 37°C and gassed with 95% O\textsubscript{2} and 5% CO\textsubscript{2}. Rings were stretched to 2 g of resting tension by means of two L-shaped stainless-steel wires inserted into the lumen and attached to the chamber and to an isometric force-displacement transducer (Letigraph 2000), respectively, as previously described.\textsuperscript{29} The concentration-relaxation response curves to ACh (10\textsuperscript{\textminus}{8}–10\textsuperscript{\textminus}{5} mol/L) were performed in rings precontracted by 10\textsuperscript{\textminus}{6} mol/L phenylephrine.\textsuperscript{29} The concentration-relaxation response curves to nitroprusside (10\textsuperscript{\textminus}{10}–10\textsuperscript{\textminus}{5} mol/L)\textsuperscript{29} were performed in the dark in rings precontracted by 10\textsuperscript{\textminus}{6} mol/L phenylephrine. In some rings without endothelium, a concentration-response curve to norepinephrine (10\textsuperscript{\textminus}{9}–10\textsuperscript{\textminus}{6} mol/L) or angiotensin II (10\textsuperscript{\textminus}{10}–10\textsuperscript{\textminus}{6} mol/L) was carried out by cumulative addition of the drugs.

To evaluate the formation of basal NO, the contraction induced by 30 minutes of incubation with the NOS inhibitor NG-nitro-L-arginine methyl ester (L-NAME) (10\textsuperscript{\textminus}{4} mol/L)\textsuperscript{29,30} was measured in ring segments precontracted with phenylephrine to approximately 40% of maximum contraction.

**Western blotting analysis**

Aortic homogenates were run on sodium dodecyl sulfate polyacrylamide electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes, incubated with primary monoclonal mouse anti-eNOS or anti–caveolin-1 antibodies (Transduction Laboratories, San Diego, CA) or polyclonal goat anti–caveolin-1 or rabbit anti–angiotensin II (AT-1) receptor (Santa Cruz Biotechnology, Santa Cruz, CA) and with the corresponding secondary peroxidase-conjugated antibodies. Antibody binding was detected by an electrochemiluminescent system (Amersham Pharmacia Biotech, Amersham, UK), and densitometric analysis was performed using Scion Image-Release Beta 4.02 software (http://www.scioncorp.com).\textsuperscript{30} Samples were reprobed for expression of smooth muscle \(\alpha\)-actin.

**In situ detection of vascular O\textsubscript{2}\textsuperscript{\textminus} production**

Unfixed thoracic aortic rings were cryopreserved (phosphate buffer solution 0.1 mol/L, phosphate-buffered saline, plus 30% sucrose for 1-2 h), included in optimum cutting temperature compound media, and frozen (−80°C), and 10-\textmu m cross sections were obtained in a cryostat (Microm International model HM500 OM). Sections were incubated for 30 minutes in 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid–buffered solution containing dihydroethidium (DHE) (10\textsuperscript{\textminus}{5} mol/L) for 30 minutes, counterstained with the nuclear stain 4′,6-diamidino-2-phenylindole 2 HCl (DAPI), and examined on a fluorescence microscope (Leica DM IRB, Wetzlar, Germany). Sections were photographed, and ethidium and DAPI fluorescence were quantified.
using ImageJ (version 1.32j, National Institutes of Health, http://rsb.info.nih/ij). \(O_2^-\) production was estimated from the ratio of ethidium/DAPI fluorescence.\(^{30}\) In preliminary experiments, DHE fluorescence was almost abolished by the \(O_2^-\) scavenger tiron, indicating the specificity of this reaction.

### Plasma nitrite plus nitrate

To measure both plasma nitrite and nitrate concentration (NOx), deproteinized plasma samples were incubated with nitrate reductase to reduce nitrates to nitrites. The final concentrations of NOx were determined by adding Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine in 5% orthophosphoric acid) to the samples and measuring the absorbance at 560 nm.\(^{29}\)

#### Statistical analysis

Results are expressed as mean ± SEM of measurements. Statistical analysis was performed by a one-way analysis of variance followed by a Newman-Keuls test. Because most of the parameters analyzed could not be measured at baseline (except body weight and blood pressure, which were not statistically significant among groups), all statistical comparisons were performed at the end of the study period. \(P < 0.05\) was considered statistically significant. The primary endpoint of the study was to assess changes in endothelial function (ex vivo relaxation to ACh). Most other parameters were measured to analyze the potential mechanisms involved in the alteration of endothelial function. Given the number of parameters measured and compared among groups (secondary endpoints), we cannot exclude that some study findings might be related to chance.

### RESULTS

#### Blood pressure, morphological variables and plasma estrogens, and NOx

Systolic blood pressure did not differ between OVX and sham-operated SHRs. Both long-term genistein and 17β-estradiol administration induced a progressive reduction in systolic blood pressure (10% at the end of the 5 weeks, \(P < 0.05\) vs untreated OVX SHRs; Table 1).

Final body weight was increased in OVX placebo-treated SHRs compared with sham-operated rats and lowered by treatment with 17β-estradiol but not by genistein (Table 1). Uterine weight, a long-term parameter of ER activation, was lower in OVX placebo-treated compared with sham-operated rats, and increased substantially by treatment with 17β-estradiol but not by genistein. Ovariectomy also led to a decreased absolute and relative kidney weight compared with sham-operated rats, and this was prevented by 17β-estradiol but not by genistein. Absolute left ventricle weight and left ventricle weight relative to heart weight, an index for left ventricular hypertrophy, were similar in all four groups of SHRs. Thus, changes in left ventricle weight relative to body weight (Table 1) seem to reflect changes in body mass rather than true changes in ventricular hypertrophy.

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**TABLE 1. Systolic blood pressure, body and organ weights, and cardiac and renal indices**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline SBP (mm Hg)</th>
<th>Final SBP (mm Hg)</th>
<th>BW (g)</th>
<th>HW (mg)</th>
<th>LVW (mg)</th>
<th>KW (mg)</th>
<th>LVW/HW ratio</th>
<th>LVW/BW ratio</th>
<th>KW/BW ratio</th>
<th>UW (mg)</th>
</tr>
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<tbody>
<tr>
<td>Sham-placebo</td>
<td>204 ± 2</td>
<td>202 ± 2</td>
<td>228 ± 5</td>
<td>910 ± 19</td>
<td>684 ± 16</td>
<td>700 ± 18</td>
<td>0.75 ± 0.01</td>
<td>3.00 ± 0.05</td>
<td>3.07 ± 0.06</td>
<td>234 ± 11</td>
</tr>
<tr>
<td>OVX-placebo</td>
<td>197 ± 2</td>
<td>199 ± 2</td>
<td>252 ± 4</td>
<td>891 ± 16</td>
<td>671 ± 12</td>
<td>631 ± 9</td>
<td>0.75 ± 0.01</td>
<td>2.67 ± 0.04</td>
<td>2.51 ± 0.03</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>OVX-genistein</td>
<td>198 ± 1</td>
<td>178 ± 2(^{ab})</td>
<td>261 ± 6(^a)</td>
<td>927 ± 16</td>
<td>703 ± 11</td>
<td>667 ± 16</td>
<td>0.76 ± 0.01</td>
<td>2.70 ± 0.05</td>
<td>2.56 ± 0.04</td>
<td>57 ± 3</td>
</tr>
<tr>
<td>OVX-estradiol</td>
<td>198 ± 2</td>
<td>178 ± 2(^{ab})</td>
<td>212 ± 7(^b)</td>
<td>913 ± 32</td>
<td>689 ± 29</td>
<td>680 ± 20</td>
<td>0.75 ± 0.01</td>
<td>3.25 ± 0.11</td>
<td>3.22 ± 0.07</td>
<td>170 ± 13(^{ab})</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of eight rats. SBP, systolic blood pressure; BW, body weight; HW, heart weight; LVW, left ventricle weight; KW, kidney weight; UW, uterus weight; OVX, ovariectomized.

\(^a\)\(P < 0.05\) compared with sham group.

\(^b\)\(P < 0.05\) compared with OVX-placebo.

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**FIG. 1.** Endothelium-dependent relaxation induced by acetylcholine (ACh) (A), endothelium-independent relaxation induced by sodium nitropresside (SNP) (B), and endothelium-dependent contractions induced by the nitric oxide synthase inhibitor L-NAME (C) in aortas from sham (n = 8), ovariectomized (OVX) placebo (n = 8), OVX genistein (n = 8), and OVX estradiol (OVX E\(_2\), n = 8) groups. ACh-, SNP-, and L-NAME–induced responses were analyzed in arteries contracted by phenylephrine. \(*P < 0.05\) vs sham group, \(^\#P < 0.05\), and \(^{\#\#}P < 0.01\) vs OVX placebo group.
Estradiol plasma levels were markedly reduced in OVX SHRs treated with placebo or genistein (undetectable) compared with sham-operated animals (25.7 ± 3.7 pg/mL, n = 4) and normalized by 17β-estradiol treatment (29.7 ± 3.6 pg/mL, n = 4). NOx was also reduced in plasma from OVX SHRs compared with sham-operated rats (6.9 ± 0.9 μmol/L [n = 8] and 22.7 ± 5.9 μmol/L [n = 8], respectively; P < 0.05). Both genistein and 17β-estradiol treatments significantly (P < 0.05) increased plasma NOx in OVX animals (16.7 ± 3.8 μmol/L [n = 8] and 29.2 ± 7.8 μM [n = 8], respectively).

Ex vivo aortic reactivity

ACh induced a relaxant response that was abolished by endothelium removal, as described elsewhere. Endothelium-dependent relaxation was significantly reduced in aortas from OVX rats compared with sham-operated rats (Fig. 1A). The endothelium-independent relaxation induced by sodium nitroprusside was not different between groups (Fig. 1B). Treatment with either genistein or 17β-estradiol significantly augmented the ACh-induced relaxation of aortic rings from OVX rats (Fig. 1A) without affecting the response to sodium nitroprusside (Fig. 1B).

In rings preconstricted with phenylephrine to approximately 40% of its maximal response, the NOS inhibitor L-NAME induced a significantly higher contraction in aortas from sham-operated than from OVX animals (Fig. 1C), indicating a reduced basal NO formation in OVX SHRs. L-NAME–induced contraction in aortic rings from OVX SHRs was significantly augmented by either genistein or 17β-estradiol, suggesting greater NO formation in these vessels.

No differences were observed in the vasoconstrictor responses induced by norepinephrine between sham-operated and OVX rats (Fig. 2A). However, the contractions induced by angiotensin II were greater in OVX than in sham-operated rats (Fig. 2B). Treatment with either genistein or 17β-estradiol significantly reduced angiotensin

FIG. 2. Effect of genistein on aortic vasoconstriction in endothelium-denuded rings from sham (n = 8), ovariectomized (OVX) placebo (n = 8), OVX genistein (n = 8), and OVX estradiol (OVX E2, n = 8) groups. Vasoconstriction of aortic rings was evoked by norepinephrine (A) and angiotensin II (B). Values are expressed as mean ± SEM. *P < 0.05 vs sham group, †P < 0.05 vs OVX placebo group.

FIG. 3. Western blots of endothelial nitric oxide synthase (eNOS) (A), caveolin-1 (Cav-1) (B), and calmodulin-1 (C) protein expression in aorta from sham (S), ovariectomized (OVX) placebo (OP), OVX genistein (OG), and OVX estradiol (OE) groups. Samples were reprobed for expression of α-skeletal actin. Results are presented as protein abundance/α-actin ratio, and comparisons with this ratio in aorta from sham-operated rats on the same gels, expressed as 100%. Densitometric analysis was expressed as mean ± SEM (four per group). *P < 0.05 vs sham group, †P < 0.05 vs OVX placebo group.
II–induced contraction of aortic rings from OVX SHRs (Fig. 2B) without affecting the response to norepinephrine (Fig. 2A).

Expression of eNOS, caveolin-1, calmodulin-1, and AT-1 receptor in rat aorta

eNOS protein expression was unchanged in OVX rats compared with sham-operated rats (Fig. 3A). We next examined changes in expression of caveolin-1, an allosteric negative regulator of eNOS, and calmodulin-1, which positively regulates eNOS activity. The expression of caveolin-1 was markedly greater in aortas from OVX SHRs than sham-operated rats (Fig. 3B) without changes in calmodulin-1 protein expression (Fig. 3C). After treatment of OVX SHRs with genistein or 17β-estradiol for 5 weeks, eNOS protein expression was unchanged (Fig. 3A), whereas caveolin-1 was reduced (Fig. 3B) and calmodulin-1 was increased (Fig. 3C).

AT-1 protein expression was increased in OVX rats compared with sham-operated rats (Fig. 4). Treatment with either genistein or 17β-estradiol normalized AT-1 protein expression in aortas from OVX rats.

In situ detection of $O_2^-$ production in rat aorta

To characterize and localize $O_2^-$ production within the vascular wall, ethidium red fluorescence was analyzed in sections of aorta incubated with DHE. DHE is oxidized by $O_2^-$ to yield ethidium, which stains DNA. Positive red nuclei could be observed in adventitial, medial, and endothelial cells (Fig. 5A). Nuclear red ethidium fluorescence, indicative of $O_2^-$ production, was quantified and
normalized to the blue fluorescence of the nuclear stain DAPI, allowing comparisons between different sections. Rings from OVX SHRs showed marked increased staining in adventitial, medial, and endothelial cells compared with sham-operated rats, which was restored by genistein or 17β-estradiol (Fig. 5A, B).

DISCUSSION

This study shows for the first time that genistein reduces blood pressure, improves endothelial function, and reduces angiotensin II contractile responses in large vessels in a model of hypertension and estrogen deficiency. It is also the first study to show that genistein-induced improvement of endothelial function was associated with reduced caveolin-1 and AT-1 receptor expression, increased calmodulin-1 and unchanged eNOS expression, and reduced vascular superoxide production. All the vascular effects of genistein paralleled those of the natural estrogen 17β-estradiol, but in contrast to 17β-estradiol, genistein had no uterotrophic effect.

The most characteristic feature of endothelial dysfunction is a diminished bioactivity of endothelium-derived NO. In the rat aorta, endothelium-dependent vasodilatation relies almost entirely on the endothelial release of NO. The SHR is a well-known and widely used animal model of endothelial dysfunction, which is aggravated after ovariectomy, resembling that observed in postmenopausal hypertensive women. More specifically, OVX SHRs showed a reduced relaxant response to ACh, an endothelium- and NO-dependent vasodilator, and an unchanged response to nitroprusside, an NO donor that relaxes arteries in an endothelium-independent manner. In addition, aortic rings from OVX rats showed a reduced contraction in response to l-NAME, an inhibitor of eNOS. Furthermore, plasma levels of NOx (nitrite plus nitrate, ie, the stable metabolites of NO), which provide an indicator of NOS activity, were reduced in OVX rats. Taken together, these data indicate that OVX rats show endothelial dysfunction characterized by a reduced NO bioactivity. The isoflavone genistein and the natural estrogen were able to restore the endothelium-dependent vasodilator response to ACh without affecting the response to nitroprusside and increased both the contraction evoked by l-NAME and plasma NOx levels.

Potential mechanisms whereby ovariectomy decreased endothelium-derived NO responses and its reversal by genistein and estradiol may be related to (1) changes in the activity and/or expression of eNOS, (2) changes in the vascular levels of O$_2^-$ and thus O$_2^-$-driven NO inactivation, and (3) changes in the sensibility to the NO–cyclic guanosine monophosphate pathway in vascular smooth muscle cells. Because the responses to nitroprusside were not modified by ovariectomy or the treatments, the third potential mechanism can be ruled out. Reduced NO synthesis associated with endothelial dysfunction may be caused by impaired expression of eNOS, posttranscriptional modification of the enzyme (eg, phosphorylation or fatty acid modifications), interactions with heat shock protein 90 and caveolin, or suboptimal concentrations of the substrate l-arginine or the cofactor tetrahydrobiopterin (BH4). In agreement with Wassmann et al, but in contrast with Widder et al, we found that expression of eNOS in aorta from SHRs was not altered by ovariectomy or estrogen therapy. eNOS activity is inhibited by caveolin-1. Cell stimulation with Ca$^{2+}$-mobilizing agonists promotes calmodulin binding to eNOS and caveolin dissociation from the enzyme, rendering the enzyme active. Xu et al reported increased caveolin-1 expression and reduced NO-dependent dilation after ovariectomy in cerebral arterioles. Accordingly, in SHRs we found that ovariectomy led to an increased protein expression of caveolin-1, which was restored by estradiol. In addition, estrogen treatment produced an increase in calmodulin expression (positive regulator of eNOS). Therefore, these changes in the expression of eNOS modulators are consistent with the widely observed endothelial dysfunction after ovariectomy and its restoration by estrogen therapy. Interestingly, the effects of genistein paralleled those of estradiol, reducing the expression of caveolin-1 and increasing calmodulin, suggesting a common mechanism for the natural estrogen and the phytoestrogen. In addition, O$_2^-$ rapidly inactivates NO and is a major contributor to the onset and development of endothelial dysfunction. Herein, we show that ovariectomy led to increased O$_2^-$ production in the vessel wall, which was prevented by genistein and estradiol, an additional mechanism helping to explain the changes in endothelial function.

AT-1 receptor activation induces vasoconstriction and cellular growth and leads to free radical release in the vessel wall. It has been reported that estrogen causes down-regulation of vascular AT-1 receptors and that estrogen deficiency is accompanied by AT-1 receptor overexpression. In OVX SHRs, genistein and estradiol prevented AT-1 receptor overexpression. Moreover, OVX SHRs showed increased vasoconstriction induced by angiotensin II, and this increase was inhibited by both drugs. In contrast, the vasoconstriction induced by norepinephrine was unchanged by either ovariectomy or the drugs. These results indicate that changes in AT-1 receptor expression show a clear functional correlate, ie, parallel changes in angiotensin II vasoconstriction. The reduced AT-1 receptor expression is also consistent with the decrease in O$_2^-$ production and the increased endothelial function. In fact, AT-1 receptor antagonists improve endothelial function in SHR. The mechanisms involved in the reduced expression of AT-1 receptor induced by chronic genistein remain unclear. These effects might be mediated by ER activation or by peroxisome proliferator-activated receptor-γ activation. This latter mechanism has been recently shown to be involved in the inhibitory effects of in vitro genistein on the expression of AT-1 receptors and superoxide production in aortic endothelial cells from stroke-prone SHRs. Alternatively, tyrosine kinase inhibition or the direct antioxidant effect of genistein may also be
involved. It is also interesting to note that angiotensin II modulates caveolin-1 expression, and thus it is tempting to speculate that expression changes in caveolin-1 after ovariectomy, estradiol, and genistein may be secondary to different AT-1 receptor activity.

Notably, genistein and 17β-estradiol showed overlapping effects with regard to vascular function. In contrast, although 17β-estradiol modifies uterine weights, the beneficial effects of genistein on blood pressure and endothelial function were not accompanied by any change in the uterine weight. The lack of a uterotropic effect of genistein is consistent with its low affinity for ERα, which contrasts with the similar potency of 17β-estradiol on ERα and ERβ. Therefore, our results would suggest that ERβ is sufficient to explain the vascular effects of estrogens. However, we cannot exclude that part of the protective effects of genistein may involve ER-independent mechanisms. In fact, in an in vitro study in arteries from male SHRs, we found that the acute (20 min) treatment with genistein enhanced endothelium-dependent vasodilatation, which was not prevented by a high-affinity ERα and ERβ pure antagonist ICI 182,780. Plasma genistein concentrations at 2 hours after a dose of 20 mg/kg body weight, double that used in our study, were approximately 11 μmol/L, and the plasma estradiol concentration in the present study was 0.11 μmol/L. Because estradiol is between 10 and 150 times more potent than genistein in activating ERβ and ERα, respectively, we believe that our dosing scheme for genistein and estradiol is comparable.

CONCLUSION

These data demonstrate that long-term genistein treatment reduced the elevated blood pressure, endothelial dysfunction, and hyperactivity of angiotensin II in large vessels from O VX SHRs to the same extent as estrogen repletion. This effect seems to be related to the increased NO bioactivity, resulting from reduced caveolin-1 and increased calmodulin-1 protein expression, presumably increasing NOS activity, and by the reduced AT-1 receptor expression and vascular superoxide anion production, presumably reducing NO inactivation. The present findings may help to explain the potential benefits of genistein as a therapeutic agent for preventing the menopausal vascular complications, especially in hypertensive women.

REFERENCES

27. Bermejo A, Zarzuelo A, Duarte J. In vivo vascular effects of genistein


