Estrogen promotes microvascular pathology in female stroke-prone spontaneously hypertensive rats

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Submitted 21 January 2003; accepted in final form 26 March 2003

Stier, Charles T., Jr., Praveen N. Chander, Louis Rosenfeld, and C. Andrew Powers. Estrogen promotes microvascular pathology in female stroke-prone spontaneously hypertensive rats. Am J Physiol Endocrinol Metab 285: E232–E239, 2003. First published April 1, 2003; 10.1152/ajpendo.00029.2003.—Estrogen produces both beneficial and adverse effects on cardiovascular health via mechanisms that remain unclear. Stroke-prone spontaneously hypertensive rats (SHRSP) maintained on Stroke-Prone Rodent Diet and 1% NaCl drinking water (starting at 8 wk of age) rapidly develop stroke and malignant nephrosclerosis that can be prevented, despite continued hypertension, by drugs targeting angiotensin II and aldosterone actions. This study evaluated estrogen’s effects in the SHRSP model. Female SHRSP that were sham operated (SHAM), ovariectomized (OVX) at 4 wk of age, or OVX and treated with estradiol benzoate (E2, 30 μg·kg⁻¹·wk⁻¹) were studied. In a survival protocol, OVX rats lived significantly longer (15.1 ± 0.3 wk) compared with SHAM (13.6 ± 0.2 wk) or OVX+E2 rats (12.4 ± 0.2 wk). In a protocol in which animals were matched for age, at 11.5 wk, terminal systolic blood pressure and urine protein excretion were elevated in SHAM and OVX+E2 rats compared with OVX rats; blood urea nitrogen, renal microvascular and glomerular lesions, and plasma renin concentration were elevated in OVX+E2 relative to SHAM or OVX rats. In a survival protocol using intact female SHRSP, treatment with an antiestrogen (tamoxifen, 7 mg·kg⁻¹·wk⁻¹) prolonged survival by >2 wk compared with controls (P < 0.01). The data indicate that estrogen promotes microangiopathy in the kidney and stroke in saline-drinking SHRSP.

hypertension; ovariectomy; proteinuria; renal microangiopathy; tamoxifen

SPONTANEOUSLY HYPERTENSIVE RATS of the stroke-prone sub strain (SHRSP) develop severe hypertension, malignant nephrosclerosis, and stroke (16, 18). Strokes in SHRSP have been reported to be pathologically similar to those in humans (33). The renal lesions in SHRSP are characterized by thrombotic microangiopathy and are similar to those seen in patients with malignant nephrosclerosis. Female SHRSP maintained on a diet of conventional rat chow and water exhibit a diminished incidence of cerebral lesions and prolonged survival compared with male SHRSP (34, 36). Under these conditions, the average lifespan of male SHRSP is 33–41 wk (18, 33), whereas female SHRSP live beyond 50 wk of age. However, placing SHRSP on a 1% NaCl drinking solution and Stroke-Prone Rodent Diet greatly enhances the expression of vascular damage in both male and female SHRSP. We have previously reported that, under these dietary conditions, the incidence of stroke and cerebrovascular damage in female SHRSP is markedly accelerated, with mortality occurring between 12 and 15 wk of age (10). Although the risk of cardiovascular disease is low in premenopausal women, estrogen-containing oral contraceptives have been reported to increase the occurrence of adverse cardiovascular incidents, especially when other risk factors such as hypertension, cigarette smoking, and migraine are also present (7, 8, 14). On the basis of these observations, we hypothesized that estrogen has a role in the stroke and kidney damage that occurs in saline-drinking female SHRSP. To test this hypothesis, bilateral surgical removal of the ovaries was performed in SHRSP shortly after weaning (4 wk of age) to eliminate the effects of endogenous estrogen at an early age. A low dose of estradiol benzoate (E2) was then started at 5 wk of age to determine whether estrogen replacement would reverse the effects of ovariectomy on the development of stroke and renal pathology. In an additional experimental series, saline-drinking female SHRSP with intact ovaries were chronically treated with tamoxifen, an estrogen receptor antagonist, to determine whether this would have an effect on the development of stroke.

MATERIALS AND METHODS

Experimental animals. Experiments were performed using female SHRSP (generations F-70 to F-72) from our colony at New York Medical College. These animals were bred from National Institutes of Health stock, which were derived from the SHRSP/A3N substrain described originally by Okamoto et al. (18). Animals were weaned at 4 wk of age and then fed a standard rodent diet (Purina Lab Chow no. 5001; Ralston Purina, St. Louis, MO) and allowed tap water ad libitum unless otherwise indicated by the experimental protocols. They were housed in a room at an ambient temperature of 22 ± 1°C with a 12:12-h light-dark cycle. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication no. 86–23), and the Institutional Animal Care and Use Committee at New York Medical College approved all procedures.

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Surgical procedures. Bilateral ovariectomy was performed when the animals reached 4 wk of age, as described previously (6). Rats were anesthetized with a mixture of 30% oxygen, 67% nitrous oxide, and 3% isoflurane (Aerrane; Anaquest, Madison, WI), and the surgical sites were shaved and then disinfected with 70% ethanol. The abdominal cavity was entered through a 1-cm retroperitoneal incision, and the ovary was exposed with forceps. The site immediately rostral to the uterus was clamped with a microhemostat, and the ovary was then excised. The uterus was gently placed back inside the abdominal cavity, the muscle layer was sutured, and the skin incision was closed with wound clips. Ovariectomy took ~4 min to perform in each animal, including the time for induction of and emergence from anesthesia. The rats recovered fully within 24 h of surgery.

Protocol 1 (survival study). Three groups were studied: ovariectomized SHRSP (OVX, n = 4), sham-operated SHRSP (SHAM, n = 6), and ovariectomized SHRSP treated with estradiol benzoate (E2) that was purchased from Sigma, St. Louis, MO (E2SHAM, n = 7). E2 (30 μg/kg sc, divided among 3 doses each week) or an equal volume of vehicle (sesame oil, 200 μl) was injected three times starting at 5 wk of age. This dose of estrogen was chosen on the basis of data from previous studies showing that this regimen effectively restores uterine weight (6, 20) and can increase circulating estrogen levels to 60–250 pg/ml (15) in ovariectomized rats. At 8 wk of age, all rats were given 1% NaCl solution to drink and were fed Stroke-Prone Rodent Diet (no. 39, Zeigler Brothers, Gardners, PA) ad libitum. The latter diet has been reported to cause a higher incidence of stroke in SHRSP (35) and possesses a lower content of potassium (0.7 vs. 1.2% by weight) and protein (17 vs. 22% by weight) than the standard diet (27). Treatment was continued until animals died, which was the primary end point in this experimental series.

Protocol 2 (age-matched study). Three groups were studied: ovariectomized SHRSP (OVX, n = 13), sham-operated SHRSP (SHAM, n = 14), and ovariectomized SHRSP treated with E2 (OVX + E2, n = 9). Surgery was performed at 4 wk of age, and treatment with E2 or its vehicle was started at 5 wk of age, as in protocol 1. At 8 wk of age, all animals were given 1% NaCl to drink and were fed Stroke-Prone Rodent Diet. Body weight was measured three times each week. Systolic blood pressure (SBP) of awake rats was measured at weekly intervals by tail-cuff plethysmography. Body weight was measured at least three times each week. The hearts, kidneys, adrenal glands, and uteri of rats that were killed were removed and weighed immediately. Uteri, trimmed of all periuterine fat, were allowed to dry to constant weight.

Experimental procedures. SBP was measured using a Natsume KN 210 manometer and tachometer (Peninsula Laboratories, Belmont, CA). Rats were warmed at 37°C for 10 min and allowed to rest quietly in a Lucite chamber before tail-cuff plethysmography. After a 5-min stabilization period, an average of nine blood pressure readings was obtained from each rat.

Analytical procedures. Plasma renin concentration (PRC) was measured by assay of angiotensin I (ANG I) generated during incubation of a 25-μl plasma sample with 0.5 ml of plasma from 48-h-nephrectomized rats. ANG I was measured using RIA kits purchased from New England Nuclear (Boston, MA). Renin concentration was expressed as nanograms of ANG I generated per milliliter of plasma per hour of incubation (ng·ml⁻¹·h¹⁻¹). Blood urea nitrogen (BUN) in serum samples was measured with a colorimetric diagnostic kit (Sigma). Urinary protein was measured by the sulfosalicyclic acid turbidity method, as previously described (27).

Histological evaluation. Coronal sections of ~3 mm were cut through the kidneys, and at least three to four such blocks were embedded in paraffin. Histological sections (2–3 μm) were stained with hematoxylin and eosin and Masson’s trichrome stain. The entire midcoronal section was examined by light microscopy in a blinded fashion. Glomerular damage, when present, was characterized as the presence of either ischemic retraction of glomerular tufts, with or without appreciable mesangiolysis, and necrotizing lesions. The latter were often an extension of arteriolar coagulopathic and proliferative lesions and consisted of any one or a combination of the following: segmental to occasionally global fibrinoid necrosis, focal thrombosis of capillaries, often accompanied by swelling, and occasional proliferation of intracapillary (endothelial and mesangial) and extracapillary (crenlucent) cells, as well as edematous expansion of mesangium without significant hypercellularity. The number of glomeruli per midcoronal section exhibiting lesions was enumerated from each kidney and was expressed as a percentage of the total number of glomeruli present (mean ± SE = 222 ± 5 glomeruli counted per animal). Acute vascular damage was assessed by counting the total number of arterial and arteriolar profiles per midcoronal section showing necrotizing and proliferative arteriopathy. Necrotizing arteriopathy was characterized by fibrinoid necrosis, extravasation of red blood cells, and luminal thrombosis; proliferative arteriopathy was characterized by proliferation of markedly swollen cells with large round-to-ovoid vesicular nuclei surrounded by mucinous extracellular matrix with or without necrotizing lesions. Renal vascular lesions were expressed as the number of lesioned arteries and arterioles per 100 glomeruli.

Statistical analysis. Data for multiple observations over time were analyzed by repeated-measures analysis of variance for overall treatment effects with software package SAS (Statistical Analysis System, Cary, NC) and a Newman-Keuls test for comparison of treatment groups at different times.
When appropriate, data were transformed (square root) before analysis to normalize group variances. Some data were analyzed using unpaired t-tests. Differences between means were considered statistically significant at P < 0.05. Cumulative percent survival curves were analyzed using the BMDP software package (BMDP Statistical Software, Los Angeles, CA) for Mantel-Cox statistics.

RESULTS

Survival study. Female SHRSP that were sham operated for OVX began to show signs of stroke at 12 wk of age and died between 12.7 and 14.4 wk of age (Fig. 1). The average age at death in the SHAM group was $13.6 \pm 0.2$ wk. OVX increased longevity of SHRSP by $\sim 2$ wk (the average age at death was $15.1 \pm 0.4$ wk) relative to their sham-operated littermate controls ($P < 0.01$). E2 replacement reversed the effect of OVX to prolong survival and hastened mortality relative to SHAM SHRSP ($P < 0.01$). All animals in the OVX+E2 group showed neurological signs of stroke and died between 12.1 and 13.4 wk of age. Histological analysis of the brains revealed cerebrovascular lesions, as we have previously described (10), indicating that these animals had succumbed to strokes. There was no difference in the heart, kidney, and adrenal gland weight as a percentage of body weight among the groups (Table 1). Changes in body weight and uterine weight due to OVX or OVX+E2 treatment were consistent with prior observations (6, 20).

Age-matched study. Female SHRSP in this protocol underwent the same experimental preparation and treatment as the animals in the survival protocol, except that these animals were all killed at 11.5 wk of age, which was before the anticipated onset of mortality. The changes in body weight over time showed no difference among the groups until 6.5 wk of age (Fig. 2). Thereafter, body weight was less in the OVX+E2 group compared with the OVX group, and at 10 wk of age, the OVX+E2 group’s body weight was less than that of either the OVX or SHAM groups. OVX was associated with a significant increase in body weight compared with SHAM starting at 7.5 wk of age. SBP did not differ among the groups at 9.2 wk of age (Fig. 3). SBP was lower in OVX compared with OVX+E2 at 10.3 wk of age and lower compared with either SHAM or OVX at 11.5 wk of age. SBP was slightly but significantly higher in OVX+E2 compared with SHAM only at 10.3 wk of age.

At 11.5 wk of age, there was no significant difference in PRC or BUN between OVX and SHAM groups; however, PRC and BUN were greater in OVX+E2 than in either OVX or SHAM (Table 2). One week before animals were killed, at 10.5 wk of age, OVX+E2 exhibited proteinuria (27.7 ± 7.9 mg/day) that was four times greater than in SHAM or OVX ($P < 0.01$). At 11.5 wk of age, SHAM exhibited elevated urinary protein excretion compared with OVX, and OVX+E2 exhibited significantly greater urinary protein excretion compared with OVX or SHAM (Table 2). Urinary protein excretion remained at baseline levels in OVX.

Figure 4 shows the histological appearance of the renal cortex from the animals in these groups at autopsy. Glomeruli, arteries and arterioles, tubules, and interstitium were essentially unremarkable in OVX (Fig. 4A) except for an occasional lesion of thrombotic microangiopathy consistent with malignant nephrosclerosis. Kidneys from SHAM exhibited scattered microvascular and glomerular lesions of malignant nephrosclerosis (Fig. 4B). These consisted mostly of focally obliteratorive fibrinoid necrosis of vessel walls and the fragmentation and extravasation of erythrocytes in foci. A few glomeruli revealed ischemic retraction of capillary tufts, possibly secondary to arteriolar obliteration. All OVX+E2 SHRSP revealed extensive renal microvascular and glomerular injury, as illustrated by the photomicrograph in Fig. 4C, which was taken at the same magnification ($\times 100$) as Fig. 4, A and B. In addition to focally massive fibrinoid necrosis of vessel walls, extravasation of fragmented erythrocytes in the vessel wall was quite prominent. Many of these vessels also showed focally nodular concentric myointimal hyperplasia affecting all layers including the adventitia. Significantly greater numbers of glomeruli compared with SHAM revealed ischemic retraction of capillary tufts, and a few revealed thrombomembranous lesions similar to those seen in the microvessels. The surrounding tubes frequently showed ischemic retraction. Sclerotic mononuclear leukocyte infiltration was seen in the adjacent interstitium in the areas of microvascular injury. These findings are very similar to those seen in human malignant nephrosclerosis and in other conditions associated with thrombotic microangiopathy. The overall results for the renal histological analysis are presented in Table 2. Microvascular and glomerular injury was significantly greater in SHAM than in OVX and was significantly greater in OVX+E2 compared with either SHAM or OVX (Table 2).

Fig. 1. Survival of stroke-prone spontaneously hypertensive rats (SHRSP) in the ovariecctomized (OVX), sham-operated (SHAM), and OVX + estradiol benzoate (OVX+E2) groups. All animals were given 1% NaCl to drink and Stroke-Prone Rodent Diet ad libitum starting at 8 wk of age. Ovariectomy significantly prolonged survival of SHRSP relative to those animals that were sham-operated. Chronic E2 (30 μg/kg sc, divided among 3 doses each week) in ovariectomized SHRSP shifted the mortality curve to the left of that of the SHAM animals.
Table 1. Terminal body weight and organ weights in female stroke-prone spontaneously hypertensive rats from the survival study

<table>
<thead>
<tr>
<th></th>
<th>OVX</th>
<th>SHAM</th>
<th>OVX+E2</th>
</tr>
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<tbody>
<tr>
<td>Age at death, wk</td>
<td>15.1 ± 0.4</td>
<td>13.6 ± 0.3</td>
<td>12.4 ± 0.2</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>131.6 ± 8.2</td>
<td>117.8 ± 5.2</td>
<td>98.5 ± 3.8</td>
</tr>
<tr>
<td>Heart wt</td>
<td>Absolute, g</td>
<td>0.99 ± 0.12</td>
<td>0.86 ± 0.06</td>
</tr>
<tr>
<td>%body wt</td>
<td>0.76 ± 0.11</td>
<td>0.74 ± 0.08</td>
<td>0.78 ± 0.08</td>
</tr>
<tr>
<td>Total kidney wt</td>
<td>Absolute, g</td>
<td>1.79 ± 0.12</td>
<td>1.53 ± 0.08</td>
</tr>
<tr>
<td>%body wt</td>
<td>1.37 ± 0.10</td>
<td>1.30 ± 0.06</td>
<td>1.40 ± 0.07</td>
</tr>
<tr>
<td>Total adrenal wt</td>
<td>Absolute, mg</td>
<td>64.9 ± 3.0</td>
<td>61.8 ± 2.8</td>
</tr>
<tr>
<td>%body wt</td>
<td>0.50 ± 0.04</td>
<td>0.53 ± 0.03</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>Uterine dry wt</td>
<td>Absolute, mg</td>
<td>6.0 ± 0.8</td>
<td>34.2 ± 1.2</td>
</tr>
<tr>
<td>%body wt</td>
<td>0.044 ± 0.003</td>
<td>0.292 ± 0.014</td>
<td>0.489 ± 0.047</td>
</tr>
<tr>
<td>No. of rats</td>
<td>4</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Animals were ovariectomized (OVX), sham operated (SHAM), or ovariectomized and given estradiol benzoate (OVX+E2). OVX or SHAM was performed at 4 wk of age, and chronic treatment with E2 (30 µg·kg⁻¹·wk⁻¹ sc) or vehicle (sesame oil) started at 5 wk of age. *P < 0.05, †P < 0.01 vs. OVX; ‡P < 0.05, §P < 0.01 vs. SHAM.

Fig. 2. Body weights of OVX, SHAM, and OVX+E2 SHRSP. There was no difference in body weight among the groups until 6.4 wk of age. Body weight was greater in OVX SHRSP compared with SHAM and OVX+E2-treated SHRSP starting at 6.7 wk of age. Body weight was lower in OVX receiving E2 therapy than in the other 2 groups. Values are means ± SE. Absence of SE bars indicates that the SE falls within the data point.

Fig. 3. Systolic blood pressure (SBP) did not differ among the groups at 9 wk of age. SBP went up as a function of time in all of the groups. Thereafter, OVX SHRSP had lower SBP compared with SHAM and/or OVX+E2-treated SHRSP. Estrogen therapy in OVX animals resulted in higher SBP compared with SHAM at 10.2 wk of age. Values are means ± SE.

Fig. 4. Postmortem body weight and absolute organ weights in female SHRSP. There was a significant decrease in body weight and absolute organ weights in OVX SHRSP compared with SHAM and/or OVX+E2-treated SHRSP. Values are means ± SE. Absence of SE bars indicates that the SE falls within the data point.

Fig. 5. Survival of saline-drinking female SHRSP that were treated chronically with either tamoxifen or vehicle starting at 7.3 wk of age. All animals in the vehicle-treated group showed neurological signs of stroke and died between 12.7 and 14.3 wk of age. The average age at death was 13.2 ± 0.3 wk in vehicle-treated SHRSP. The first tamoxifen-treated SHRSP died at 13.1 wk of age, and one tamoxifen-treated rat survived much longer than the others and was killed at 23 wk of age (data not shown). Survival was significantly increased in the tamoxifen-treated group (P < 0.01, Mantel-Cox statistics). Histological examination of the brains showed evidence of cerebrovascular lesions in both groups. SBP became severely elevated as a function of time, with little difference between the groups (Fig. 6A). Body weight was also significantly lower in tamoxifen-treated SHRSP starting at 9.5 wk of age (Fig. 6B). There was no difference between the groups after 11 wk of age as the vehicle-treated rats began to show stroke signs, became debilitated, and lost body weight. Postmortem body weight (121.1 ± 7.2 vs. 115.7 ± 3.5 g) and the absolute weight of the heart (0.73 ± 0.02 vs. 0.78 ± 0.03 g), kidneys (1.33 ± 0.03 vs. 1.43 ± 0.09 g), adrenal glands (64.7 ± 4.9 vs. 60.0 ± 4.8 mg), and uterine dry weight (45.6 ± 4.3 vs. 45.4 ± 5.5 mg) did not differ between the groups.

DISCUSSION

The present results indicate a role for estrogen in promoting stroke and renal microvascular injury in saline-drinking female SHRSP. In particular, survival was prolonged, and renal vascular pathology was reduced, by OVX, which diminishes endogenous estrogen levels, or by treatment with the antiestrogen tamoxifen. Moreover, E2 replacement fully reversed the effect of OVX to diminish stroke-related mortality and renal vascular lesions in saline-drinking SHRSP. Other studies have shown a decrease in pathology associated with estrogen in SHRSP and in other stroke models (1,
Fig. 4. Representative photomicrographs (H&E × 100) from saline-drinking, 11.5-wk-old female SHRSP (A), showing essentially unremarkable renal cortex status-post-OVX without E2 replacement therapy. Note a normal interlobular-size artery (arrow).

B: an SHRSP that was sham operated for OVX shows several arterioles with circumferential fibrinoid necrosis (arrowheads) accompanied by focally extravasated and fragmented erythrocytes in two vessels (arrows). Adjacent glomeruli are generally unaffected in this area. Many of the surrounding tubules, however, show ischemic retraction (some with *).

Thrombotic microangiopathic lesions affecting glomeruli and renal microvessels were significantly greater in incidence and severity than in OVX but markedly less than in OVX + E2. A sparse mononuclear leukocytic infiltrate is scattered in the surrounding interstitium (some circled).

C: SHRSP status-post-OVX + E2 replacement therapy shows massive fibrinoid necrosis affecting small interlobular arteries and arterioles accompanied by prominent extravasated and fragmented erythrocytes and frequent concentric myointimal cellular proliferation and swelling (arrows). One of the two glomeruli in the lower right quadrant reveals ischemic collapse of the capillary tuft with mesangiolysis (g). Hyaline casts (†), indicative of proteinuria, are seen in a few of the surrounding tubules. Many other tubules show ischemic retraction or reactive epithelium (some with *).

Surrounding interstitium contains areas with several mononuclear leukocytes (some circled).

Table 2. Terminal measurements made at 11.5 wk of age in saline-drinking female stroke-prone spontaneously hypertensive rats

<table>
<thead>
<tr>
<th>Property</th>
<th>O VX</th>
<th>SH AM</th>
<th>OV X + E2</th>
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| PRC, ng ANG 1·ml⁻¹·h⁻¹                      | 20.0 ± 4.9 | 33.3 ± 7.5 | 63.3 ± 12.5
| BUN, mg/dl                                   | 11.2 ± 0.9 | 12.0 ± 0.6 | 23.7 ± 5.2
| Proteinuria, mg/day                          | 6.9 ± 0.8  | 28.9 ± 7.7 | 84.7 ± 18.0
| No. renal microvascular lesions/100 glomeruli| 1.1 ± 0.4  | 4.3 ± 1.1* | 20.9 ± 4.5
| No. glomerular lesions/100 glomeruli         | 1.1 ± 0.3  | 3.3 ± 0.9* | 11.3 ± 3.0
| Uterine dry wt Absolute, mg                  | 7.8 ± 1.0  | 76.1 ± 3.8* | 59.2 ± 2.5
| mg/g body wt                                 | 0.038 ± 0.003 | 0.433 ± 0.020 | 0.413 ± 0.017
| No. of rats                                  | 13        | 14        | 9         |

Values are means ± SE. Animals were ovariectomized (OVX), sham operated (SHAM), or ovariectomized and given E2 (OVX + E2). OVX or SHAM was performed at 4 wk of age, and chronic treatment with E2 (30 μg·kg⁻¹·wk⁻¹ sc) or vehicle (sesame oil) started at 5 wk of age. All animals were given 1% NaCl to drink and Stroke-Prone Rodent Diet ad libitum starting at 7 wk of age. Plasma renin concentration (PRC) and blood urea nitrogen (BUN) were measured in blood obtained on the day the study ended, and urinary protein excretion (proteinuria) was measured in urine collected the day before. *P < 0.05, †P < 0.01 vs. OVX; ‡P < 0.05, §P < 0.01 vs. SHAM.
3). However, an important difference is that both of those studies employed middle cerebral artery occlusion to create ischemic strokes as opposed to our studies, in which cerebral microvascular injury occurs spontaneously with consequent cerebrovascular hemorrhage and/or microinfarction, which is associated with malignant hypertension. We previously reported that ≥50% of female SHRSP exhibit neurological signs of stroke by 12 wk of age when fed Stroke-Prone Rodent Diet and given 1% NaCl to drink (10). When Enovid, an oral contraceptive preparation containing estrogen and progestin, was administered to female SHRSP fed a similar low-protein fish diet and 1% NaCl, there was an even further acceleration of lethal strokes (31). However, Enovid did not provoke cerebrovascular damage in female SHRSP fed a regular diet, even though it exacerbated the naturally occurring high blood pressure in these rats (30). These observations suggest that saline-drinking female SHRSP serve as a suitable experimental model for studying the adverse systemic vascular effects of estrogen.

Previous studies have reported that physiological doses (1- and 10-µg subcutaneous injections) of estradiol resulted in plasma levels at 1 h of 250 pg/ml (1-µg dose) and 2,000 pg/ml (10-µg dose), both of which fell to <60 pg/ml by 6 h (15). Because the rats in our study received a comparable dose (10 µg/kg three times per week by the same route), the plasma levels achieved should be within the range of the estrous cycle, ~30 pg/ml in metestrus to ~140 pg/ml in proestrus (3). We had previously reported that this replacement dose of E2 provides a robust response in terms of its uterotrophic action (6, 20). Although absolute uterine weight of OVX+E2 (at 11.5 wk) was not fully restored to the level observed in age-matched SHAM, this parameter may have been affected by debilitation related to the vascular pathology. When factored for body weight, the values for these groups were nearly identical.

Our observations may provide a correlate to those found clinically. In premenopausal women, estrogen doses supplied in oral combination contraceptives have been associated with a slight but significant increase in the risk for stroke (8). However, when other risk factors are present, the incidence of stroke is markedly increased by estrogen (14). Among women who smoke cigarettes and have migraines, oral contraceptive use increased the risk for stroke 34-fold. It is tempting to speculate that SHRSP parallel this clinical profile. These animals not only have hypertension and impaired endothelial-dependent relaxation responses (29), as observed in patients who smoke cigarettes, but they also display abnormalities in their cerebrovascular circulation, as may be observed in patients with migraine. In cross-sectional studies of postmenopausal women, estrogen doses utilized for hormone replacement therapy were found to be associated with major cardiovascular protective effects (24). The beneficial actions of estrogen were attributed to improved lipid profile, increased production of nitric oxide, decreased production of endothelin, and decreased intracellular calcium in arterial smooth muscle (2). However, estrogens are known to increase thromboembolism, and the results of the prospective Heart and Estrogen/Progestin Replacement Study (HERS) trial indicated that, among women with established coronary disease, daily use of conjugated equine estrogens and medroxyprogesterone acetate increased the risk for myocardial infarction within the first year of treatment, with decreased risk during years 3–5 (11). The results of the HERS II study indicated that this beneficial effect did not persist during additional years of follow-up and that, after 6.8 yr, hormone therapy did not reduce the
risk of cardiovascular events (9). Thus the clinical or experimental setting in which estrogen is used may be of critical importance with regard to vascular complications.

To better characterize the pathogenesis of the adverse vascular effects of estrogen in SHRSP, we performed a second study under identical experimental conditions, with the exception that animals were killed at 11.5 wk of age. This early age was selected because it was before any mortality was observed in the survival studies. In agreement with the protective action observed against the onset of stroke, OVX reduced the degree of proteinuria and renal vascular and glomerular damage that developed compared with SHAM at 11.5 wk of age. Lower SBP in OVX may have contributed to the vascular protective effect observed in these animals; however, SBP did not differ between SHAM and OVX+E2 and thus cannot account for the increased renal pathology in OVX+E2 compared with SHAM. Several other studies have also implicated a role for estrogen in the production of renal injury. In the spontaneously hypercholesterolemic Imai rat, estrogen administration aggravated the development of proteinuria and glomerular injury (17). A similar aggravating effect of estrogen has been reported in female analbuminemic rats (13) and in female obese Zucker rats (25). In our studies, estrogen fully reversed the protective effect of OVX on proteinuria and renal damage and increased the degree of proteinuria, renal damage, and elevated BUN relative to SHAM. Although we cannot be sure why OVX+E2 developed worse pathology relative to SHAM, one possibility is that endogenous progesterone, which was not replaced in our studies, may have had an effect to ameliorate the action of estrogen. Together, our observations in female SHRSP and other animal models at risk for systemic vascular disease provide evidence for a promotive action of estrogen to enhance microvascular damage.

If endogenous estrogen levels have a role to promote vascular damage in female SHRSP, interventions that not only decrease estrogen levels (OVX) but also block the actions of estrogen at its receptor (tamoxifen) may decrease the incidence of stroke in these animals. Consistent with our findings with OVX, tamoxifen prolonged survival of saline-drinking, ovariectomy-intact SHRSP. Unlike OVX, however, tamoxifen failed to lower SBP in these animals. This may relate to the fact that tamoxifen is an estrogen receptor modulator and has been shown to antagonize estrogen effects in some target tissues but to act like estrogen in others. The effect of tamoxifen, therefore, may not necessarily be identical to that of ovariectomy. Tamoxifen has been reported to exert estrogen-like effects to suppress growth in rats and, more notably, to increase the risk of thromboembolism. Thus the protective effect of tamoxifen in our present study suggests that the adverse effect of estrogen was not the consequence of its prothrombotic activity. Tamoxifen has been used clinically to prevent nephropathy in patients with lupus nephritis, an autoimmune disease with acute and/or chronic inflammation believed to be aggravated by hyperestrogenemia, and to diminish nephropathy in animal models of lupus (32). The ability of tamoxifen to reduce levels of C-reactive protein and other markers of inflammation (5) suggests that a proinflammatory effect of estrogen may be responsible for the ability of this steroid hormone to promote vascular injury in this disease.

Other mechanisms might also contribute by which estrogen may promote the development of vascular pathology in female SHRSP. Activation of the renin-angiotensin-aldosterone system would be a likely possibility on the basis of our previous studies (21, 26, 27), which were performed in male SHRSP. Consistent with this notion, PRC was significantly enhanced in OVX+E2 and slightly reduced in OVX compared with SHAM. However, this association may have been the response to, rather than the cause of, renal vascular injury in these animals. We have also found that aldosterone is a critical mediator of the development of thrombotic microangiopathy, not only in salt-loaded SHRSP (21) but also in nitro-L-arginine methyl ester/ANG II/NaCl-treated rats (23). This effect of aldosterone has been associated with a vascular inflammatory phenotype (22) and may set the stage for a vicious cycle of events in which vascular injury begets inflammation, which begets vascular injury, and may explain the malignant course of events leading to end-organ damage in SHRSP. We have not measured aldosterone levels in this experiment. Nonetheless, on the basis of high PRC in the adrenal-intact animals, we expect that aldosterone levels would also be elevated. The ability of estrogen to accelerate the progression of vascular injury under the conditions of our study may thus be due to its proinflammatory actions, either directly or via elevation in aldosterone. In postmenopausal women, hormone replacement therapy has been associated with an increase in C-reactive protein and other markers of inflammation (5). In the rat, estrogen has been reported to have proinflammatory or anti-inflammatory actions depending on the dose, duration of exposure, and the experimental conditions (4). Another possible mechanism is that E2 may promote thrombosis through its actions to decrease antithrombin III or to increase Factors VII and X (19). Previous studies have also shown that estrogen treatment stimulates the formation of a tissue factor (TF)-like procoagulant in the immature rat uterus (12). TF is thought to be involved in the local formation of thrombin, which is a potent mitogen. In addition, estrogen is known to increase plasma protein infiltration into the uterus. By analogy, an increase in vascular permeability with plasma protein influx into the vessel wall is thought to be one of the initial events in the development of microvascular lesions of thrombotic microangiopathy in SHRSP. The precise mechanism by which estrogen promotes vascular injury in saline-drinking female SHRSP will require further investigation.

In conclusion, our findings demonstrate that OVX, which diminishes endogenous estrogen levels, or chronic treatment with tamoxifen, an anti-estrogen, significantly
prolonged survival in saline-drinking female SHRSP. OVX reduced the degree of proteinuria and renal microvascular and glomerular damage that developed compared with SHAM at 11.5 wk of age. E2 replacement fully reversed the effect of OVX to diminish stroke-related mortality and ameliorate renal pathology in saline-drinking SHRSP. The present results indicate a role for estrogen in promoting stroke and renal vascular injury in saline-drinking female SHRSP.

We gratefully acknowledge the technical assistance of Sraboni Bhattacharya, Carol Eisenberg, Newton Fan, and Gagan Singh. This study was supported in part by grants from the National Institutes of Health (HL-35522 to C. T. Stier and DK-32783 to C. A. Powers), the American Heart Association New York State Affiliate (Grant-in-Aid 9859133 to C. T. Stier), and a New York Medical College Intramural Biomedical Research Grant.

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