Estrogen depletion induces NaCl-sensitive hypertension in female spontaneously hypertensive rats

ZHIWU FANG,1 SCOTT H. CARLSON,1,2 Y. F. CHEN,2 S. OPARIL,2 AND J. MICHAEL WYSS1,2
1Department of Cell Biology and 2The Vascular Biology and Hypertension Program of the Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama 35294-0019

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Fang, Zhiwu, Scott H. Carlson, Y. F. Chen, S. Oparil, and J. Michael Wyss. Estrogen depletion induces NaCl-sensitive hypertension in female spontaneously hypertensive rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R1934–R1939, 2001.—In women, arterial pressure generally increases after menopause, but several studies suggest that women who eat large amounts of plant estrogens (phytoestrogens) experience a slower rise in the incidence of postmenopausal hypertension. This suggests that both ovarian hormones (principally estrogen) and phytoestrogens may protect at least some women from hypertension. The present study tests the hypothesis that phytoestrogens blunt hypertension in estrogen-depleted female spontaneously hypertensive rats (SHR). Three-week-old ovariectomized SHR were fed one of four diets that contained basal (0.6%) or high (8%) NaCl with or without dietary phytoestrogens for 9 wk. In SHR on the basal NaCl diet, arterial pressure was unaffected by the removal of dietary phytoestrogens. In contrast, in SHR on the high-NaCl diet, arterial pressure was significantly higher in rats on the phytoestrogen-free (204 ± 4 mmHg) compared with the phytoestrogen-replete (153 ± 4 mmHg) diet. Ganglionic blockade resulted in reductions in arterial pressure that were directly related to the dietary NaCl-induced increases in arterial pressure. Together, these data indicate that dietary phytoestrogens protect ovariectomized female SHR from dietary NaCl-sensitive hypertension and that the sympathetic nervous system plays an important role in this effect. Furthermore, these results demonstrate that dietary phytoestrogens can have a major impact on the interpretation of studies into the physiological role of estrogen in females.

IN BOTH MALES AND FEMALES, hypertension is one of the primary contributors to cardiovascular disease (the leading cause of death in the United States), but there is a sexual dimorphism in the incidence of hypertension (26). Arterial pressure in males exceeds that in similarly aged women until the age of menopause, but after that age, arterial pressure in women increases rapidly and eventually equals or exceeds that in males (26). This suggests that there is an interaction between circulating estrogen and blood pressure control in women. Furthermore, compared with age-matched men, premenopausal women are resistant to the hypertensive effects of a high-NaCl diet, but following menopause, women and men display a similar incidence of salt-sensitive hypertension (36).

A similar sexual dimorphism is present in some of the most common rat models of hypertension [e.g., spontaneously hypertensive rats (SHR), Dahl NaCl-sensitive rat]. In these animals, hypertension develops more rapidly and severely in male compared with female rats (e.g., 11, 12, 37), and female rats display less pronounced NaCl-sensitive hypertension (7). Furthermore, in female Dahl salt-sensitive rats and DOCA-NaCl rats, the elimination of endogenous estrogen blunts this sexual dimorphism and accelerates the development of NaCl-dependent hypertension (12, 37). In female compared with male SHR, the hypertensive response to a high-NaCl diet is greatly decreased (7, 8), suggesting that estrogen may blunt NaCl-sensitive hypertension in female SHR. However, ovariectomy of young female SHR does not cause a large increase in blood pressure in rats on a basal or high-NaCl diet, suggesting that endogenous estrogen is not the sole contributor to the arterial pressure protection that female SHR display (8).

Recent studies suggest that plant estrogens (phytoestrogens) can have important physiological effects. The two major phytoestrogens genistein and daidzein are abundant in soy diets that are normally fed to rats and appear to be the most physiologically active phytoestrogens in mammals (6), likely due to their structural similarity with estrogen and their high affinity binding to the β-estrogen receptor (ER-β; Refs. 5, 10). Data from other laboratories indicate that phytoestrogens in the normal diet mimic some of the beneficial effects of estrogen in rats (10, 14, 22). The present study tests the hypothesis that both plant and endogenous estrogens can protect female SHR from at least one form of hypertension, i.e., NaCl-sensitive hypertension.

MATERIALS AND METHODS

Experiment 1

Three-week-old SHR (n = 8 per group, Harlan Sprague-Dawley) were ovariectomized and allowed ad libitum access to one of the four diets during a 9-wk feeding period. The two major phytoestrogens genistein and daidzein are abundant in soy diets that are normally fed to rats. The effects of menopause in women and the development of NaCl-dependent hypertension in SHR were tested in the present study. Arterial pressure in female SHR develops more rapidly and severely than in male SHR (11, 12, 37). Ovariectomy of young female SHR does not cause a large increase in blood pressure in rats on a basal or high-NaCl diet, suggesting that endogenous estrogen is not the sole contributor to the arterial pressure protection that female SHR display (8).

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to one of four diets: normal phytoestrogen-replete (PE+) diet containing either basal (0.6%; diet #8746, Teklad, Madison, WI) or high (8%; diet #5008, Teklad) NaCl, or a phytoestrogen-free (PE−) diet containing either basal (0.6%; custom diet TD86369, Teklad) or high (8%; custom diet TD86370, Teklad) NaCl. Independent assay demonstrated that the PE+ diet contained 0.06% phytoestrogen by weight and the PE− diet contained no detectable phytoestrogens (6). Rats were maintained at constant humidity (65 ± 5%), temperature (24 ± 1°C), and light-dark cycle (0600–1800, lights on) throughout the experimental protocols. All experimental procedures were conducted in accordance with institutional and National Institutes of Health guidelines and under the approval of the University of Alabama at Birmingham’s Institutional Animal Care and Use Committee.

At 10 wk of age, all rats were anesthetized with pentobarbital sodium (35 mg/kg ip), and each rat was instrumented with an implantable arterial pressure transducer/transmitter (TA11PA-C40; Data Sciences, St. Paul, MN) as described previously (8, 9, 34). The catheter was inserted into the abdominal aorta immediately caudal to the renal arteries, and the body of the transmitter was sutured to the inside of the anterior abdominal wall. The rats were allowed at least 1 wk to recover from the operation and were housed in individual cages throughout the study. Each cage was placed on a receiver panel for recording data via the Dataquest IV software system (Data Sciences). Arterial pressure, heart rate, and activity data for each rat were recorded for a 10-s period every 5 min. After a 1-wk recovery period, arterial pressure, heart rate, and activity were recorded for 2 wk.

For analysis of the circadian rhythms of mean arterial pressure (MAP) and heart rate, mean values were calculated at 60-min intervals for each diet/strain group. The data from 3 consecutive days were subjected to rhythm analysis using a nonlinear fitting program PHARMFIT, as described previously (8, 9, 34), and the “best fit” model was defined as the one with the lowest number of harmonics that had a confidence value of at least 0.05, as determined by the subprogram SYNOPS (8, 34). The MESOR (rhythm-adjusted 24-h mean), amplitude (the peak to trough of the rhythm), acrophase (time at which each component cosine function reaches its peak), and the percentage to which the computed rhythm estimates the data were determined for all groups.

**Experiment 2**

By the end of experiment 1, MAPs of the ovariectomized SHR on the PE−, high-salt diet were extremely high, with some of the animals exceeding 260 mmHg. Therefore, we conducted a second experiment in which arterial pressure and heart rate were measured at an earlier age. Three-week-old female SHR (n = 56, Harlan Sprague-Dawley) were ovariectomized or received sham surgery (i.e., anesthetized and skin cut open; intact controls), and they were placed on one of four diets as described above (i.e., 8 groups). At 7 wk of age, all rats were again anesthetized, and each rat was instrumented with catheters implanted in the right femoral artery and vein. Three days later, arterial pressure and heart rate were monitored by connecting the arterial catheter to a pressure transducer connected to a personal computer (BioPac, Santa Barbara, CA). MAP was measured at 0200–0400 and 1400–1600, time points that approximate the nighttime peak and daytime nadir of the 24-h arterial pressure rhythm as observed in experiment 1.

To test the role of the sympathetic nervous system in the arterial pressure response to the high-NaCl diet, we blocked sympathetic nervous system activity using the ganglionic blocker hexamethonium (10 mg/kg iv) at 0200–0400, the approximate peak of the arterial pressure circadian rhythm in SHR. Arterial pressure was recorded 30 min before hexamethonium infusion and for 20 min following the treatment.

**Statistical analysis.** All data from both experiments were evaluated by analysis of variance (between-group MAP and heart rate comparisons) or repeated-means analysis of variance (within- and between-group comparisons of the hexamethonium responses) followed by appropriate post hoc tests (Newman-Keuls) to determine the source of main effects and interactions. The significance criterion for all experiments was P < 0.05.

**RESULTS**

**Experiment 1: 8-Wk Diet Exposure**

In 12-wk-old ovariectomized SHR on basal NaCl, PE+ diet, the 24-h average MAP was 130 ± 2 mmHg (Fig. 1). The removal of phytoestrogens from the basal NaCl diet (PE− group) was associated with no significant increase in MAP [136 ± 3 mmHg, not significant (NS); Fig. 1]. Exposure to the high-NaCl diet for 8 wk (from 3 wk of age) increased MAP to 153 ± 4 mmHg in the PE+ rats (an increase of 23 mmHg; P < 0.05 vs. either group fed the basal NaCl diet). The removal of phytoestrogen greatly exacerbated the MAP response to the high-NaCl diet, i.e., MAP increased to 204 ± 4 mmHg in the PE− group (an increase of 68 mmHg; P < 0.05 vs. all other groups; Fig. 1; all statistical comparisons were equally significant whether the means of the arterial pressure were generated directly from the raw data or from the individual MESORs). The high-NaCl diet led to a similar increase in amplitude of the 24-h pressure rhythm in both PE+ (4.38 mmHg) and PE− (2.77 mmHg, NS; Fig. 1), and it did not alter the acrophase of the rhythm (time of peak).

In SHR fed the basal NaCl diet, the 24-h average heart rate was significantly lower in PE+ compared

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*Fig. 1. Twenty-four-hour circadian rhythm (PHARMFIT analysis) of arterial pressure in ovariectomized spontaneously hypertensive rats (SHR) fed either a basal or high-NaCl diet with (PE+) or without (PE−) dietary phytoestrogens. The high-NaCl diet elevated arterial pressure in the PE− group, and removal of dietary phytoestrogens exacerbated the arterial pressure response to NaCl. Elimination of phytoestrogens did not significantly affect arterial pressure in rats fed the basal NaCl diet. *P < 0.05 for MESOR compared with that of all other groups. MAP, mean arterial pressure.
with PE− rats (339 ± 2 vs. 358 ± 3 beats/min, respectively; *P < 0.05; Fig. 2). Exposure to the high-NaCl diet resulted in a slight but significant reduction (−9 beats/min; *P < 0.05) in 24-h average heart rate in the PE+ rats, whereas heart rate slightly increased in the PE− group (+9 beats/min; *P < 0.05; Fig. 2). At the end of the study, there were no significant differences in body weight between the groups.

**Experiment 2: 4-Wk Diet Exposure**

Because MAP was extremely high in the high-NaCl, PE− group in experiment 1, and because no nonovariectomized rats were included in experiment 1, arterial pressure responses to the high-NaCl diet were tested at a shorter exposure to the diets (4 wk on diet) in both ovariectomized and intact rats. Because rat telemetry probes cannot reliably be implanted in animals weighing <150 g, a tethered catheter system was used for experiment 2. Body weights were significantly greater in the PE− rats (210 ± 3 g) compared with the PE+ group (191 ± 5 g; *P < 0.05), although exposure to the 8% NaCl diet did not significantly alter the body weights. However, animals on the PE− diet (207 ± 4 g) tended to be heavier than those on the PE+ diet (194 ± 4 g; NS).

**Nighttime measurement.** To assess MAP during the rat’s active period, arterial pressure was measured from 0200 to 0400 (the approximate peak of the arterial pressure circadian rhythm in SHR). Intact, 7-wk-old female SHR on basal salt, PE+ diet displayed a nighttime average blood pressure of 126 ± 4 mmHg, which was not significantly altered by removal of phytoestrogens from the diet (121 ± 1 mmHg; Fig. 3). Ovariectomy had no effect on average nighttime blood pressure in either the PE+ (121 ± 4 mmHg) or the PE− (126 ± 3 mmHg) group fed the basal NaCl diet (Fig. 3).

Exposure to the high-NaCl diet for 4 wk increased nighttime MAP in the intact PE+ group to 135 ± 3 mmHg (+9 mmHg; *P < 0.05) and in the intact PE− group to 144 ± 3 mmHg (+23 mmHg; *P < 0.05; Fig. 3). In ovariectomized PE+ rats, the high-salt diet increased nighttime MAP to 165 ± 5 mmHg (+44 mmHg; *P < 0.05 compared with all intact groups) and in the ovariectomized PE− rats to 190 ± 4 mmHg (+62 mmHg; *P < 0.05 compared with intact rats fed the same diet).

**Daytime measurement.** Arterial pressure was also measured from 1400 to 1600, the approximate nadir of the arterial pressure rhythm in SHR. Blood pressures were lower during the daytime than nighttime in all groups, and there were no significant differences in daytime MAP among the four groups fed the basal NaCl diet for 4 wk (Fig. 3). The high-NaCl diet induced a small increase in daytime MAP in the intact PE+ rats (10 mmHg; NS) and a significantly larger increase (25 mmHg; *P < 0.05) in the intact PE− group (Fig. 3). In the ovariectomized rats, the high-NaCl diet increased daytime MAP similarly in the PE+ and PE− groups (21 and 24 mmHg, respectively; Fig. 3).

**Sympathetic blockade.** To assess the contribution of sympathetic nervous system to arterial pressure, autonomic blockade was induced using hexamethonium during the approximate peak of the arterial pressure circadian rhythm in SHR (0200–0400). Acute hexamethonium blockade of sympathetic nervous system activity significantly reduced nighttime arterial pressure in all groups. The decreases correlated directly with...
the basal MAP and were the greatest in the ovariectomized PE− group fed the high-NaCl diet (Fig. 4). The hexamethonium blockade eliminated the significant nighttime arterial pressure differences between the groups.

**DISCUSSION**

The present study demonstrates that dietary phytoestrogens protect ovariectomized SHR from dietary NaCl-sensitive hypertension. In ovariectomized SHR on a normal (PE+) diet, excess dietary NaCl causes a modest increase in arterial pressure, even when the NaCl challenge is initiated as early as 3 wk of age. However, in this model, the removal of phytoestrogens from the diet greatly exacerbates the hypertensive response to a high-NaCl diet. The results of the present study also indicate that the sympathetic nervous system contributes to the exaggerated response of arterial pressure to dietary NaCl.

These findings are consistent with evidence suggesting that circulating estrogen blunts hypertension and cardiovascular disease in human females. After menopause, arterial pressure rises, resulting in a significantly higher rate of hypertension (42), including NaCl-sensitive hypertension (36), greatly increasing the risk of cardiovascular disease and mortality (36). The present study demonstrates that circulating estrogen reduces NaCl sensitivity in female SHR, although it has little effect on arterial pressure in SHR fed a basal NaCl diet.

The present findings also indicate that dietary phytoestrogens significantly reduce the increase in arterial pressure in response to dietary NaCl. These results support a growing body of evidence that indicates that both dietary phytoestrogens and endogenous estrogen have a cardioprotective effect. It is well documented that Asian (compared with Western) women have significantly lower rates of cardiovascular morbidity and mortality; the postmenopausal rise in blood pressure occurring in women from Western societies is significantly greater compared with Japanese women (1), who consume much higher levels of dietary phytoestrogens (24, 25, 39). These observations suggest that dietary phytoestrogens can mimic some of the effects of estrogen replacement therapy. Dietary phytoestrogens exert their actions primarily through ER-α and ER-β (18, 27, 28, 35), but their protective effects may also be mediated through their ability to inhibit tyrosine kinase (23). Research suggests that phytoestrogens preferentially bind to ER-β (vs. ER-α) receptors. Genistein, the primary phytoestrogen in soy, has negligible binding affinity for the ER-α receptor but binds to ER-β receptors with only three times lower affinity than 17β-estradiol (27). Thus we hypothesize that the anti-hypertensive effects of phytoestrogens are mediated by the ER-β receptor, leading to alterations in vascular compliance (2, 33, 46), renal function (40), and circulating lipid levels (17), without inducing breast or uterine cancer (20, 21), the most serious potential side effects of estrogen replacement therapy (3, 8, 21). However, clinical studies have not convincingly demonstrated a beneficial effect of phytoestrogen treatment in women (15, 19).

The means by which estrogen and phytoestrogens protect female SHR from NaCl-induced changes in blood flow are unclear but probably include both peripheral and central nervous system mechanisms. Estrogen’s vasoprotective effects include its ability to blunt vasoconstrictor responses to norepinephrine by reducing intracellular free calcium (4) and to enhance vasodilation by increasing nitric oxide release (13). Similar effects have been reported in response to phytoestrogen consumption (2, 33, 46). Estrogen and phytoestrogens may also act centrally to reduce sympathetic activity (43) and to increase parasympathetic activity (30) by modulating baroreflex function and/or altering brain control of arterial pressure (41). In the present study, heart rate was slightly higher in PE− rats fed the basal NaCl diet compared with the PE+ group. Furthermore, in contrast to PE+ rats, PE− rats failed to reduce heart rate following exposure to the high-NaCl diet. These observations are consistent with the hypothesis that hypertension and NaCl-sensitive hypertension in SHR are due, at least in part, to overactivity of the sympathetic nervous system (9, 31, 47). We more formally tested this hypothesis by using hexamethonium to block sympathetic outflow during the peak period of the arterial pressure rhythm. The greatest decrease in arterial pressure in response to the hexamethonium challenge occurred in the ovariectomized SHR on the high-NaCl, PE− diet, whereas the second largest drop was found in the ovariectomized rats fed the high-NaCl, PE+ diet. Furthermore, treatment with hexamethonium reduced blood pressure in both groups to similar levels. These results indicate that the sympathetic nervous system contributes to the NaCl-sensitive hypertension in estrogen-depleted rats. Moreover, our data suggest that both endogenous and exogenous estrogens blunt the sympathetic nervous system response to the high-NaCl diet, which is consistent with other evidence indicating that female sex...
hormones may reduce circulating catecholamine levels (32) and suppress sympathetic nervous system activity and hypertension in male SHR (16).

The present study demonstrates that after a 2-mo exposure to a high-NaCl, PE− diet, blood pressure increases significantly during both nighttime and daytime periods in ovariectomized SHR. In contrast, at an earlier time point (1 mo after exposure to the diet), nighttime (but not daytime) arterial pressure is significantly increased. This is similar to our findings in male SHR in which a high-NaCl diet increases nighttime arterial pressure much sooner than it increases daytime arterial pressure (8). Thus, in both male and estrogen-depleted female SHR, a high-NaCl diet causes an eventual increase in daytime arterial pressure, leading to a decreased amplitude of the arterial pressure circadian rhythm (8). The failure of arterial pressure to dip during the daytime in estrogen-depleted female SHR chronically fed a high-NaCl diet (8) is similar to the failure of arterial pressure to decrease during sleep periods in many established hypertensive humans, i.e., nondippers (38, 44, 45) appear to accelerate both cardiovascular and renal disease (29, 38, 44, 45).

**Perspectives**

The present results demonstrate that dietary phytoestrogens protect female SHR from NaCl-sensitive hypertension. Although a high-NaCl diet normally causes only a modest increase in arterial pressure in intact female SHR, removal of either endogenous ovarian estrogen or exogenous dietary phytoestrogens elicits NaCl-sensitive hypertension. The most important finding of the present study is that the depletion of both endogenous and exogenous estrogen dramatically exacerbates the hypertensive effect of a high-NaCl diet. Furthermore, this protective effect of both estrogen sources appears to be related to their ability to prevent sympathetic nervous system overactivity in response to a high-NaCl diet. These data suggest that research examining the biological role of estrogen should carefully consider the similar and synergistic actions that endogenous and exogenous estrogens may produce. They also suggest that the role of estrogens should carefully examined in postmenopausal women who are under stress, whether that be dietary or emotional.

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**REFERENCES**