Estrogen Depletion Increases Blood Pressure and Hypothalamic Norepinephrine in Middle-Aged Spontaneously Hypertensive Rats

Ning Peng, John T. Clark, Chi-Chang Wei, J. Michael Wyss

Abstract—In male spontaneously hypertensive rats (SHR) a high NaCl diet increases arterial pressure via a reduction in anterior hypothalamic nucleus norepinephrine release. Young female SHR are relatively well protected from this NaCl-sensitive hypertension, but depletion of both endogenous and dietary estrogens greatly exacerbates NaCl-sensitive hypertension. This study tests the hypothesis that estrogen also protects late middle-aged female SHR from NaCl-sensitive hypertension and that this effect is mediated by an estrogen-related effect on hypothalamic norepinephrine release. Ten-month-old female SHR were ovariectomized and placed on a phytoestrogen-free diet containing either basal or high NaCl. Each rat was implanted with a silastic tube containing 17β estradiol or vehicle. Three months later, arterial pressure and hypothalamic norepinephrine metabolite levels (MOPEG) were measured. On the basal NaCl diet, estrogen-depleted rats displayed increased arterial pressure (12 mm Hg) and decreased anterior hypothalamic nucleus MOPEG (20%). Both effects were reversed by estrogen treatment. In all groups, the high NaCl diet increased arterial pressure by over 35 mm Hg and reduced anterior hypothalamic nucleus MOPEG by >60%. Across all groups, there was a significant inverse correlation between arterial pressure and anterior hypothalamic nucleus MOPEG. These data suggest that both dietary NaCl excess and estrogen depletion raise arterial pressure in middle-aged female SHR by a decreasing hypothalamic norepinephrine. (Hypertension. 2003;41:1164-1167.)

Key Words: estrogen • norepinephrine • sodium • sympathetic nervous system • blood pressure

Hypertension significantly contributes to premature death in the fastest-growing segment of the population, ie, adults over 65 years of age. The incidence of hypertension in this age group is about 60%. Further, between 30% and 50% of hypertensive patients are NaCl-sensitive, ie, they display a significant (>10%) increase in blood pressure when placed on a high NaCl diet (200 to 250 mEq Na+/d for 7 days). The NaCl-sensitive hypertensive patients, in contrast to normotensive and NaCl-resistant hypertensive subjects, fail to suppress plasma norepinephrine (NE) after the ingestion of a diet high in NaCl. These and other studies suggest that the sympathetic nervous system contributes importantly to NaCl-sensitive hypertension in aging humans.

Several lines of evidence indicate that, compared with men, premenopausal women have a lower rate of hypertension. After menopause this relative protection appears to be lost, and women experience a rapid rise in hypertension (including salt-sensitive hypertension). This increase appears to be related to the menopausal loss of estrogen. Our studies in spontaneously hypertensive rats (SHR) demonstrate that young female SHR (compared with age-matched male SHR) have a lower arterial pressure and a reduced hypertensive response to excess dietary NaCl. Elimination of most endogenous estrogen by ovariectomy causes no significant change in arterial pressure and only a modest alteration in NaCl-sensitive hypertension. However, simultaneous removal of both endogenous and dietary estrogens (phytoestrogens in soy-based rodent chow) leads to a small rise in baseline arterial pressure and a very large increase in the hypertensive response to a high NaCl diet.

Further, in male SHR, a high NaCl diet activates the sympathetic nervous system and, thereby, increases arterial pressure. A key gender difference in the response to the dietary NaCl excess is that in male (but not female) SHR, the high NaCl diet decreases norepinephrine release in an important sympato-inhibitory nucleus, the anterior hypothalamic nucleus (AHN). The present study further tests the hypothesis that, in female SHR, the loss of estrogen allows dietary NaCl excess to decrease norepinephrine (NE) release in AHN and, thereby, leads to increased NaCl-sensitive hypertension. We are particularly interested in understanding how estrogen protects females during the perimenopausal period, because studies have demonstrated that estrogen treatment during this period can significantly reduce later cardiovascular events.
Our studies demonstrate that female SHR begin to stop estrous cycling around 8 months of age and from that time onward display constant plasma estrogen levels typical of diestrous. Further, at this age, their cardiovascular system has been subjected to challenge from hypertension and associated circulating factors. Thus, we used middle-aged SHR in this study, so as to better model the effects of estrogen in a perimenopausal human.

Methods

Experiments were performed in conscious, freely moving female SHR (Harlan Sprague-Dawley, Inc, Indianapolis, Ind). At 10 months of age, the rats (under pentobarbital anesthesia) were ovariectomized or sham operated and implanted (under the skin of the flank region) with a Silastic tube that was either filled with estradiol (estradiol-treated) or unfilled (vehicle control) and incubated overnight in 0.01 mol/L phosphate-buffered saline.15 The Silastic capsules were replaced every 25 days with newly filled implants. The rats were then placed on diets that were devoid of phytoestrogens (AIN76A, Teklad) containing either 8% NaCl (high NaCl diet) or 0.6% NaCl (basal NaCl diet; n=8/group). Animals were maintained on a 12/12-hour light/dark cycle (light from 06:00 to 18:00) at a constant temperature (24±1°C) and humidity (60±5%), and they were housed 3 rats per cage before surgery.

Three months later (at 13 months of age), all rats were anesthetized with sodium pentobarbital (50 mg/kg, IP), and a stainless steel guide cannula was stereotaxically implanted above the AHN.15,16 Five days after implantation of the guide cannula, the animals were anesthetized with Brevital (50 mg/kg, IP), and catheters were implanted in the abdominal aorta for measurement of arterial pressure.17 On the next day, a push-pull assembly was inserted into the guide cannula, so that its tip extended 1 mm beyond the guide cannula, and push-pull monitoring was initiated.16,17 After a 30-minute equilibration period, perfusate was collected in 10-minute fractions in plastic tubes containing 2.5 mL of 0.5 N perchloric acid-EDTA solution (0°C), and mean arterial pressure (MAP) was monitored for 90 minutes. At the conclusion of the experiment, 0.3% pontamine sky-blue dye in artificial cerebrospinal fluid (ACSF) was perfused for 3 minutes, and the animal was sacrificed with an overdose of ether. The brain was removed and sectioned for histologic verification of cannula placement by an investigator who was blind to the group designation of the sections examined. Blood was collected for subsequent measurement of serum estradiol concentration. Serum estradiol levels were determined by radioimmunoassay with commercially available kits (Diagnostic Products Corporation; assay sensitivity=0.5 pg/mL for estradiol). Because of a technical error, 18 of the estradiol measures were lost, and the final averages were collapsed across the diet groups (>8 samples/group). Monoamines and metabolites in the perfusate were measured using high performance liquid chromatography with electrochemical detection (HPLC-EC) as described previously.6,7

Of the 48 rats in the study, 9 were eliminated for the following reasons: (1) cannula placement was outside the AHN (1 basal intact, 1 basal vehicle, and 1 high salt estrogen-treated), (2) failure of the blood pressure monitoring (1 basal estrogen treated, 2 high salt intact), (3) premature death (2 high salt vehicle and 1 high salt estrogen-treated). Thus, the final groups each included at least 6 animals, and each of the basal groups included 7 rats.

Statistics

The results are expressed as mean±SEM. Because no alterations in conditions were made to the animals during the nine 10-minute baseline collections of the microperfusate, the mean of each animal’s AHN 3-methoxy-4-hydroxy-phenylglycol (MOPEG) and MAP were entered into the analysis. Analysis included a 2-way analysis of variance with appropriate post hoc tests (Newman-Keuls) to determine the source of the effects. In each animal, the standard error of the mean for the MOPEG and MAP for the 9 sampling time points were approximately 5% of the mean values, and there was no tendency for these values to increase or decrease in a time-dependent manner in any group. Significance was statistically defined as P<0.05.

Results

Estrogen depletion and treatment effectively altered serum estradiol concentration and uterine size. At the end of the experiment, the average plasma estradiol concentration in the intact rats was 26±11 pg/mL. Plasma estradiol concentration in the ovariectomized SHR that were treated with 17β estradiol was 66±9 pg/mL and was nondetectable (<0.5 pg/mL) in the vehicle-treated SHR. We did not evaluate estrus cyclicity in the intact rats. Numerous studies indicate that, at this age, female rats no longer display cycles.18,19 Our own archival data indicate that 10- and 15-month-old virgin female SHR display constant estrus. At autopsy, the uterus was nearly undetectable in vehicle-treated ovariectomized rats (0.11±0.03 g), but it was of normal size in the intact (0.54±0.02 g) and estradiol-treated, ovariectomized rats (0.60±0.04 g).

At 13 months of age, after 3 months of treatment, arterial pressure in the SHR was significantly affected by both estrogen (F[2,33]=10.9, P<0.05) and dietary NaCl (F[1,33]=107, P<0.05; interaction F[2,33]=2.5, NS). In the SHR on the basal NaCl diet, removal of dietary estrogen and ovariectomy resulted in a 12 mm Hg rise in arterial pressure (Figure 1; P<0.05), and treatment with 17β estradiol prevented this rise. The 3-month exposure to the high NaCl diet elevated arterial pressure 40 mm Hg in the gonadally intact group and 49 mm Hg (P<0.05; Figure 1) in the ovariectomized group. Estradiol replacement blunted the NaCl-induced rise in arterial pressure in the ovariectomized rats.

Heart rate was not significantly altered by the estrogen manipulations in the rats on the high or basal NaCl diet. However, the high NaCl diet significantly raised baseline heart rate in intact (330±5 to 389±16 bpm), ovariectomized (320±6 to 416±15 bpm), and estradiol-replaced (329±9 to 396±13 bpm) groups. Body weights were initially similar in the 6 groups (227±1 g). In both high and basal NaCl-fed rats, estrogen depletion resulted in a body weight increase (37 g and 39 g, respectively), and estrogen treatment caused a slight body weight decrease in the basal and high NaCl-fed rats (24 g and 18 g, respectively; P<0.05). The intact rats showed no
significant change in body weight. All animals appeared to be healthy and groomed normally.

Baseline MOPEG in the AHN was significantly altered by both dietary NaCl ($F_{2,33} = 211, P < 0.05$) and estrogen status ($F_{2,33} = 10.9, P < 0.05$; interaction $F_{2,33} = 1.9, \text{NS}$), and was correlated closely with arterial pressure ($r = 0.84$; Figures 2 and 3). On a basal NaCl diet, estrogen depletion reduced basal AHN MOPEG by about 24%, and 17β-estradiol treatment returned AHN MOPEG to approximately the concentration of intact controls (Figure 2). The 8% NaCl diet lowered AHN MOPEG in all 3 groups by over 60% (compared with their counterparts on controls (Figure 2). The 8% NaCl diet lowered AHN MOPEG in all 3 groups by over 60% (compared with their counterparts on controls (Figure 2). Estrogen depletion further decreased AHN MOPEG, and estrogen treatment returned it to the intact control level (Figure 2).

**Discussion**

These results demonstrate that middle-aged female SHR respond to a high NaCl diet with a relatively large rise in arterial pressure. Chronic estrogen depletion modestly increases this NaCl-sensitive arterial pressure response. The high NaCl diet also decreases AHN MOPEG, and this reduction, which is inversely correlated with arterial pressure, is exacerbated by chronic estrogen depletion.

These results demonstrate responses to dietary NaCl excess and estrogen that are rather different from those in young female SHR. Our previous studies demonstrate that young adult, female SHR are protected from dietary NaCl-sensitive hypertension by estrogen. In 3-month-old female SHR, dietary NaCl excess causes a modest rise in arterial pressure (≈10 mm Hg), but chronic estrogen depletion greatly exacerbates the hypertensive response to a high NaCl diet (>40 mm Hg). Compared with these data from young SHR, the current data indicate that estrogen is much less protective in older SHR. Whereas the NaCl-induced rise in arterial pressure in the estrogen-depleted SHR in the current study is ≈50 mm Hg, the rise in the estrogen-replete (intact) and the estrogen-treated groups is nearly 40 mm Hg (Figure 1).

One potentially confounding factor in this analysis is that arterial pressure is very high in middle-aged SHR on the high NaCl diet. Thus, the rise in arterial pressure that is induced by the combination of estrogen depletion and a high NaCl diet may be blunted by a ceiling effect, ie, it may be difficult to chronically raise arterial pressure much above a MAP of 190 mm Hg in these rats. Further, the larger NaCl-induced arterial pressure responses in the middle-aged (compared with young) intact and estrogen-treated rats may be due to a longer (2 vs 3 months) exposure of the rats to a high NaCl diet. Also, estrogen may simply be more effective in protecting the vessels and cardiovascular control mechanisms in young rats, in which the cardiovascular system has been exposed to hypertension (and thereby damaged) for a relatively shorter time. In the middle-aged rats, resistance vessels and control mechanisms have been stressed by hypertension for >8 months before the exposure to excess dietary NaCl, and at that point, the antihypertensive regulatory elements may no longer be capable of responding effectively to the dietary NaCl challenge. It is of interest to note that the reported beneficial effects of hormone replacement therapy appear to be related to early “perimenopausal” treatments, and the effectiveness of this therapy significantly diminishes if it is not initiated until later in menopause. Alternatively, the lessened responsiveness in older SHR may reflect an impairment of their estrogen response mechanisms, eg, reduced estrogen receptors.

The results of this study suggest that the mechanisms that underlie NaCl-sensitive hypertension in female middle-aged SHR are, at least in some respects, similar to the mechanisms that underlie NaCl-sensitive hypertension in young male SHR. In young male SHR, the ability of a high NaCl diet to increase arterial pressure is dependent on the sympathetic nervous system. A 2-week exposure of young male SHR to a high NaCl diet increases arterial pressure, elevates plasma norepinephrine, and decreases norepinephrine concentration in AHN, but not in other brain regions. Further, our studies in male SHR demonstrate that changes in AHN MOPEG concentration reflect altered norepinephrine release, as opposed to reuptake. These and other results indicate that dietary NaCl loading increases blood pressure in male SHR by reducing norepinephrine release in AHN, thus removing an important sympatho-inhibitory mechanism that normally modulates arterial pressure. Other studies indicate that a high NaCl diet elevates plasma sodium, which in turn activates osmo-receptive neurons in the circumventricular hypothalamic nuclei. The activation of these neurons thereby suppresses norepinephrine release from axon terminals in AHN, probably via an indirect pathway that includes an inhibitory neuromodulator. Further, in SHR males a continuous infusion of an adrenergic receptor agonist into the AHN blocks the hypertensive effects of a high NaCl diet, and lesions of the AHN raise arterial pressure but block dietary NaCl-sensitive hypertension in young male SHR. The current

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**Figure 2.** A bar graph to demonstrate the MOPEG concentration in the AHN of female SHR in each group. *P < 0.05 compared with groups on the same diet; †‡P < 0.05 compared with all 3 basal NaCl-fed groups.

**Figure 3.** A scatter graph to demonstrate the correlation ($r = 0.84$) between mean arterial pressure and AHN MOPEG in the female SHR groups.
results suggest that NaCl-sensitive hypertension in middle-aged female SHR may be related to a similar NaCl-induced reduction in norepinephrine in the AHN and that estrogen depletion may elevate NaCl sensitivity through this same neural mechanism.

Although further studies are needed to clarify the role of the AHN in NaCl-sensitive hypertension in this model, other mechanisms are also likely to be involved in the cascade leading to NaCl-sensitive hypertension in these rats. Renal function is impaired in SHR, and this may contribute importantly to potential sodium and volume loading in the high NaCl-fed rats. Also, our preliminary study indicates that the elimination of phytoestrogens from the diet of male SHR increases their hypertensive response to a high NaCl diet by a sympathetic nervous system mechanism, thus suggesting that phytoestrogens are protective in male SHR.

Perspectives

The protective role that estrogen plays in the cardiovascular system of females appears to be complex. Young female SHR are resistant to the hypertensive effects of a high NaCl diet, but estrogen depletion greatly increases their responsiveness. In contrast, middle-aged female SHR display a marked rise in arterial pressure when challenged with a high NaCl diet, and this sensitivity is only moderately increased by estrogen depletion. In the middle-aged female SHR, the high NaCl diet decreases AHN norepinephrine to about the same extent as it does in young males, suggesting that this form of hypertension in middle-aged female SHR may also be mediated by a neuronal mechanism that includes decreased norepinephrine release in AHN, i.e., the middle-aged female appears to take on a male-like NaCl-sensitive phenotype. This suggests that prolonged exposure to hypertension and age-related alterations in estrogen release may greatly affect the interaction among the nervous system, estrogen, dietary NaCl, and hypertension.

Acknowledgment

This work was funded by grants HL 37722 and NS 41071 (J.M.W., Nong, nervous system, estrogen, dietary NaCl, and hypertension. This work was funded by grants HL 37722 and NS 41071 (J.M.W., Nong, nervous system, estrogen, dietary NaCl, and hypertension.

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Hypertension. 2003;41:1164-1167; originally published online March 24, 2003;
doi: 10.1161/01.HYP.0000065387.09043.2E
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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