Estrogen Ameliorates N$^{\omega}$-nitro-L-arginine Methyl Ester-induced Blood Pressure Increment in Male Spontaneously Hypertensive Rats: the Role of cGMP

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Abstract

Estrogen (17beta-estradiol, or E_2) reduces systolic blood pressure (SBP) increment and increases aortic cyclic guanosine monophosphate (cGMP) in male spontaneously hypertensive rats (SHRs). It is unknown, however, whether the E_2-enhanced aortic cGMP is essential for the BP-lowering effect or not. N$^{\omega}$-nitro-L-arginine-methyl ester (L-NAME), an L-arginine analogue and nitric oxide (NO) synthase inhibitor, significantly increases SBP and decreases aortic cGMP in male SHRs. We thus treated male SHRs with vehicle (corn oil) or E_2 (s.c, 2 mg/kg/week) with or without L-NAME (20 mg/dl in the drinking water). SBP was measured weekly. Plasma nitrate/nitrite (NOx) concentrations and aortic cGMP levels were all measured at the end of the study. We found that SBP increment was significantly higher in L-NAME group, compared with the controls, and that E_2 treatment reduced this L-NAME effect. Plasma NOx concentrations were not significantly different among different groups. Basal and acetylcholine-induced aortic cGMP, but not sodium nitroprusside-induced cGMP, were significantly lower in L-NAME group, compared with the controls. E_2 co-administration did not modify L-NAME-induced aortic cGMP decrease. These data indicate that E_2-induced BP-lowering effect in L-NAME treated male SHRs is not closely associated with the enhancement of vascular cGMP.

Key Words: blood pressure, estradiol, nitric oxide, cyclic guanosine monophosphate, spontaneously hypertensive rats.

Introduction

The incidence of hypertension is accelerated in postmenopausal women (6, 27, 31). Estrogen supplement induces a favorable lipid change or a significant reduction of blood pressure value in postmenopausal women (1, 26), and ovariectomized (13, 29) as well as intact female animals (14, 32). In addition to the females, accumulative evidence has supported that estrogen (E_2) administration also exerts beneficial effects on cardiovascular system in the males. E_2 administration improves the lipid profile i.e., increased high-density lipoprotein and decreased low-density lipoprotein) in healthy elder males (11), reduces total peripheral resistance in male normotensive rats (4) and lowers blood pressure (BP) increment in hypogonadal males (20) and in spontaneously hypertensive male rats (SHRs) (14,38). E_2 also restores endothelium-mediated shear stress-induced relaxation in arterioles of male SHRs (15). Furthermore, E_2 attenuated the course of the deoxycorticosterone-salt hypertension in male rats (7), suggesting that not only genetic hypertension is affected.

A negative correlation exists between systolic blood pressure (SBP) and aortic cyclic guanosine monophosphate (cGMP) in male normotensive (3)

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and in SHRs (9). In addition, an increase in aortic cGMP is associated with the E_2-induced BP-lowering effect in male SHRs (38). However, it is unknown whether the enhanced aortic cGMP is necessary for E_2's BP-lowering effect.

N^{ω}-nitro-L-arginine-methyl ester (L-NAME), a L-arginine analogue and nitric oxide (NO) synthase inhibitor, significantly increases SBP and decreases aortic cGMP in male normotensive (3, 35) and in hypertensive rats (2, 24). In addition, L-NAME-induced SBP increment is more pronounced in SHRs than in normotensive rats (2). Therefore, L-NAME-treated male SHRs would be an ideal model to test whether E_2-induced BP-lowering effect is dependent on a high vascular cGMP.

To test the effect, male SHRs were administered with L-NAME (20 mg/dl dissolved in drinking water) with and without E_2 co-administration (2 mg/kg/week, s.c, respectively). We found that E_2 co-administration reduced the L-NAME-induced SBP increment, but it did not reduce the L-NAME-induced aortic cGMP decrease in male SHRs. Therefore, we suggested that E_2-induced BP-lowering effect in L-NAME-treated male SHRs was not dependent on the enhancement of vascular cGMP.

Materials and Methods

Animals

Male SHRs were obtained from the National Science Council and maintained at the Animal Center of Chang Gung University. Fifteen-week-old SHRs with similar body weights (about 275 g) and systolic blood pressures (SBP) were randomly divided into three groups: control group, L-NAME group and L-NAME plus E_2 group. Both control and L-NAME groups had subcutaneous administration of cholesterol-free corn oil (1 ml/kg) once a week for 3 weeks. The L-NAME group received a daily intake of about 24. 6 mg/kg with L-NAME (roung/dl) dissolved in drinking water for 2 weeks. The L-NAME plus E_2 group received subcutaneous administration of E_2 at the dosage of 2 mg/kg once a week for three weeks, and a daily intake of about 18.8 mg/kg with L-NAME (20 mg/dl) dissolved in drinking water for two weeks. E_2 and L-NAME administration began in the 15th and 16th weeks, respectively. The dosage (20 mg/dl) of L-NAME used in this study is based on a previous observation, which shows that from 10 to 100 mg/kg/day L-NAME administration, there are similar increments in blood pressure and decreases in aortic cGMP contents (3). Heart rate and SBP was recorded weekly by tail-cuff method as described previously (37) in 15- to 18-week olds. At the end of the experiment, rats were anesthetized by pentobarbital (50 mg/kg, i.p.) and then the aorta as well as whole blood samples were collected. All experimental procedures were performed in accordance with the approved institutional protocols.

Plasma Nitrite/Nitrate Contents Measurement

Blood collected from heart was centrifuged (Kubota 5100, 3,000 rpm, 10 min) to obtain plasma and then the plasma was centrifuged (Kubota 1700, 14,000 rpm, 20 min) through a 30-KDa cut-off filter (YM-30, Microcon) to reduce the background absorbance due to the presence of hemoglobin. The clear centrifuge was collected to determine the nitrite/nitrate contents using Nitrite/Nitrate Colorimetric Assay Kit (Cayman, Ann Arbor, MI, USA).

Aortic Cyclic GMP Contents Measurement

Descending aorta was isolated from rats, removed from connective tissues and cut into aortic ring (2 mm in length) in Krebs solution (1 ml) with the composition (in mM) of 118.4 NaCl, 25 $NaHCO_3$, 11.66 glucose, 4.75 KCl, 1.18 $MgSO_4 \cdot 7H_2O$, 2.5 $CaCl_2 \cdot 2H_2O$, 1.19 KH_2PO_4, 0.02 EDTA maintained at pH 7.4 and aerated with 95%O_2-5%CO_2 at room temperature. After incubation with 3-isobutyl-1-methylxanthine (0.5 mM) for 3 min, aortic rings were treated with 10 μM acetylcholine (ACh) for 1 min, or 1 μM sodium nitroprusside (SNP) for 1.5 min. Krebs solution was then removed and aortic rings were frozen in liquid nitrogen. These frozen aortic rings were homogenized in 6% trichloroacetic acid (TCA) and then the homogenate was centrifuged (Kubota 1700, 14,000 rpm, 20 min) to collect the clear supernatant. The supernatant was extracted with 4-volume water-saturated diethyl ether for six times to remove TCA, and then the aqueous extract was lyophilized by speed-vac. The dried extract dissolved in assay buffer was used to assess the cGMP contents using cGMP [^{125}I] assay kit (Amersham Pharmacia Biotech, UK).

Statistical Analysis

All values are expressed as mean±S.E.M. Differences between mean values of two groups were analyzed by Student's *t* test. All comparisons were computed using GraphPad InStat 2.0 program (GraphPad software, CA). Significance was accepted at $P < 0.05$.

Results

Reduction of L-NAME-Induced SBP Increase by E_2

The SBP values were similar at the beginning

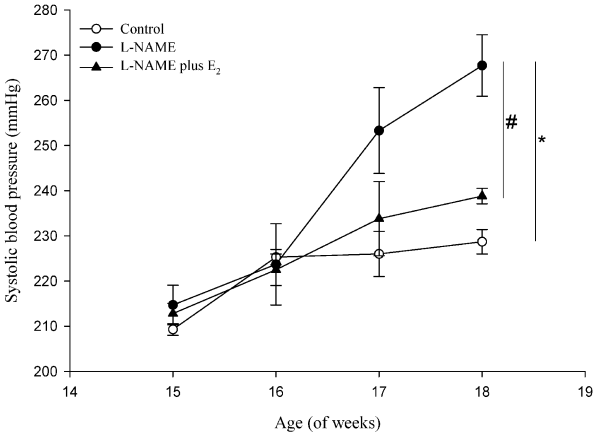

Fig. 1. SBP development in control and L-NAME-treated rats with or without E_2 co-administration. E_2 (2 mg/kg once a week, s.c) and L-NAME (20 mg/dl in the drinking water) administration began at week 15 and 16, respectively. All values are presented as mean±S.E.M., n=4 for all groups. *P < 0.05 between control (open circle) and L-NAME-treated rats (closed circle); # P < 0.05 between L-NAME-treated rats with or without E_2 co-administration (closed triangle).

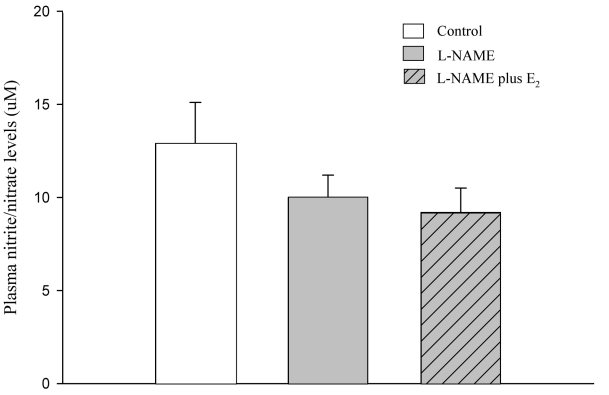

Fig. 2. Plasma nitrite/nitrate concentration in control and L-NAME-treated rats in the absence or presence of E_2 co-administration at week 18. All values are presented as mean±S.E.M., n=4 for all groups.

in the control and L-NAME group (16 wk, about 223 mmHg). After a 2-week treatment of L-NAME, the SBP of L-NAME group significantly increased by 44.0±4.4 mmHg (19%) (Fig. 1). The SBP of the control group did not change significantly during this period. L-NAME administration also significantly increased SBP in the presence of E_2 by 16.3±3.1 mmHg (7%), but the L-NAME-induced SBP increment was significantly lower in E2-treated rats (P=0.003).

Effect of L-NAME on Plasma Nitrite/Nitrate Levels

Plasma nitrite/nitrate (NOx) levels were not significantly different among all groups (Fig. 2).

Decrease of Aortic cGMP Levels Is not Modified by E_2 in L-NAME-treated Rats

Figure 3A shows that the basal aortic cGMP concentration was significantly reduced by L-NAME, regardless of the supplement of E_2 or not. Acetylcholine stimulated cGMP concentration slightly and L-NAME inhibited the aortic cGMP concentration significantly in the absence or presence of E_2 (Fig. 3B). However, sodium nitroprusside (SNP) greatly stimulated aortic cGMP concentration to a similar extent among these three groups (Fig. 3C).

Discussion

We found that L-NAME administration significantly increased SBP development (Fig. 1) and decreased basal aortic cGMP (Fig. 3A) in male SHRs.

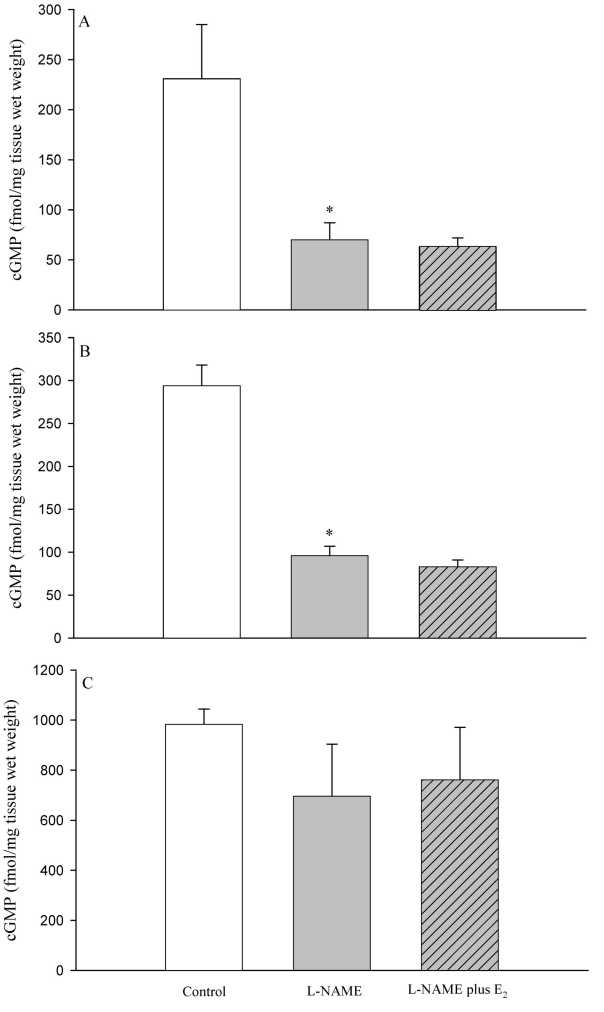

Fig. 3. Basal (A), acetylcholine (B)- and sodium nitroprusside (C)-induced aortic cGMP levels in control and L-NAME-treated rats in the absence or presence of E_2 co-administration at week 18. All values are presented as mean±S.E.M., n=4 for all groups. *P < 0.05 between control (open column) and L-NAME-treated rats (closed column).

Co-administration of 17beta-estradiol (E_2) significantly reduced the L-NAME-induced increment in SBP (Fig. 1); however, it did not modify the L-NAME-induced decrease in basal and acetylcholine-induced aortic cGMP (Fig. 3A and 3B). Thus, E_2 exerts BP-lowering effect in L-NAME-treated male SHRs with low vascular cGMP concentration.

E_2-induced BP-lowering effect in L-NAME-treated male SHRs was reported previously (38) and that co-administration of E_2 (about 0.44 mg/kg/week) prevents L-NAME (about 160 mg/kg/day)-induced SBP increment in the pregnant Sprague-Dawley rats (5). In addition to the L-NAME-treated rats, E_2 also exerts BP-lowering effect in ovariectomized rats (13) and transgenic (mRen2) 27 hypertensive rats (23) as well as hypogonadal men (20). However, not all of the E_2 studies illustrated the BP-lowering effect (16, 18).

Low dose of L-NAME (about 20 mg/kg/day) did not modify plasma nitrite/nitrate (NOx) concentration in this study (Fig. 2). Similar result was observed in male normotensive rats with low (20 mg/kg/day, similar to our dosage) (25) or high dose (100 mg/kg/day) (40) of L-NAME administration. However, most studies utilizing a range of L-NAME from 50 to 100 mg/kg/day demonstrate significantly reduced plasma NOx level (3,25). The reason for the discrepancy at higher dose of L-NAME remains unknown.

Although low dose of L-NAME administration did not alter plasma NOx concentration, it significantly reduced basal and ACh-induced aortic cGMP in SHRs (Fig. 3A and 3B). This result is consistent with the finding that aortic cGMP is significantly reduced in normotensive (3) and New Zealand genetically hypertensive rats (24) with low dose (10 mg/kg/day) of L-NAME administration.

Our results indicated that E_2's BP-lowering effect could be dissociated from aortic cGMP level in L-NAME-treated male SHRs. Interestingly, quinapril (angiotensin I-converting enzyme inhibitor) prevented the BP increment in L-NAME-treated male normotensive rats without increasing aortic cGMP (12). Thus, although a negative correlation between BP and aortic cGMP has been reported (3,10), BP increment in L-NAME-treated rats can be reduced either by E_2 (Fig. 1) or drug (12) in a cGMP-independent manner. Furthermore, cGMP-dependent protein kinase G (PKG) activity or concentration is not affected by E_2 (33) or L-NAME (2), suggesting that cGMP/PKG-dependent pathway would not completely explain the E_2-induced BP-lowering effect in L-NAME-treated rats. These findings reflect that the vascular actions of E_2 are diverse, at least partly due to the complex subtype/signaling of estrogen receptor (ER) in transmitting the E_2 actions (21). For example, E_2 inhibits the vascular injury response in ERα-deficient mice as well (17) suggesting mechanism independent of classic ER. Earlier report (36) has also indicated that E_2 activates voltage-gated K^+ channel through a cGMP-dependent mechanism in coronary arteries. Taken together, on the one hand, E_2 might exert various vascular effects *via* ER-dependent but genomic-independent and cGMP-independent pathways (21); on the other hand, relaxation *via* ion channel activation on vascular smooth muscle cells might also be mediated *via* cGMP. Thus, E_2 has multiple effects in addition to cGMP-dependent pathway on cardiovascular system (26) and hypertension (8). E_2 stimulated the opening of calcium-activated potassium channels *via* cGMP-dependent (36) or independent (39) pathway. In addition, E_2 treatment decreased reactive oxygen species productions (22,34) and plasma concentrations of rennin angiotensin (30), while both were increased in L-NAME-treated rats (19,28).

These results suggested that in addition to NO-cGMP pathway, other mechanism(s) might likely be involved in the E_2-induced BP-lowering effect in L-NAME-treated male SHRs.

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