Short Communication

Gender-dependent Response in Blood Pressure Changes Following the Inhibition of Nitric Oxide Synthase

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Abstract

Many studies employed L-NAME (N^G-nitro-L-arginine methyl ester), an L-arginine antagonist and nitric oxide (NO) synthase (NOS) inhibitor, to produce hypertension experimentally in male animals. It is not known whether females respond similarly. We thus examined the effect of long-term oral administration of L-NAME on body weight (BW), blood pressure (BP), and heart rate (HR) of both female and male rats. We found that L-NAME induced significant increase in mean BP (MAP) in both genders, however, L-NAME-treated females (F*) exhibited a significantly higher elevation than males (M*) did. This difference persisted for 5 wks and then diminished. L-NAME was thus withdrawn and a rapid decrease of MAP was observed. MAP of F* decreased less and thus remained higher than M* for 5 wks. MAP of control rats (F and M) remained unchanged during the period. Systolic BP (SBP) altered in a similar pattern. We also found that HR decreased immediately after L-NAME administration and that HR of F* was significantly less reduced. These findings indicate that L-NAME induced a more pronounced response in females than males, consistent with the view that females are more dependent on NOS activity for their regulation of BP.

Key Words: hypertension, nitric oxide, sexual dimorphism, L-NAME

Introduction

The L-arginine/nitric oxide/cyclic GMP (L-Arg/NO/cGMP) pathway has been established as one of the important regulatory systems of blood pressure (1-4). By inhibition of NO synthase with L-Arg analogues such as N^G-nitro-L-arginine methyl ester (L-NAME), blood pressure exhibits marked increase in both human and animal (3,4). Although many studies attempted to illustrate the significance and potential mechanisms involved in the L-NAME-induced hypertension, most were performed in males (3-9). It is not known whether gender difference exists in these chronic NO-deficient hypertension. However, gender difference in the cardiovascular functions and diseases is well known (10-12) including the observations that premenopausal women have lower risk for hypertension and that estrogen replacement therapy is effective in reducing risks in postmenopausal women (10). Recently, evidence indicates that estrogen receptor (ERβ)-deficient mice develop sustained hypertension with vascular dysfunction (13). Considering that estrogen up-regulates endothelial NO synthase (14,15), L-NAME treatment is likely to exert different effects on males and females. We thus examined the chronic effects of L-NAME on blood pressure, heart rate, and body weight in conscious rats of both sexes as a preliminary study. Following 10 weeks of treatment, L-NAME was withdrawn and observations continued for another 7 weeks to examine whether gender difference occurred during the withdrawal phase.

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Materials and Methods

Wistar-Kyoto (WKY) rats (4wks of age) were obtained from the National Science Council and kept in the Animal Facility of Chang Gung University for 2 wks. They were divided into 4 groups: male control (M), female control (F), L-NAME-treated males (M*) and females (F*). Twenty four WKYs with similar baseline values of mean BP (MAP), systolic BP (SBP), and heart rate (HR) were used for this study. They were measured weekly by tail-cuff method (UR 5000, UEDA, Japan) as described previously (16). Body weight (BW), food, and water uptake were determined twice a week. Due to the apparent differences in BW between M and F (also M* and F*, see Fig. 1), L-NAME dose (dissolved in drinking water at about 20 mg/100mL) was calculated and adjusted every week according to the weekly water uptake (27.6 ± 1.2 mL for M and M*, 26.0 ± 0.9 mL for F and F* at 7th wk) and body weight. The average intakes of L-NAME were 45.3 ± 0.9 mg/kg wk and 42.6 ± 1.2 mg/kg wk for M and F, respectively at the beginning (7th wk). There was no significant difference in L-NAME uptake between genders. The treatments were carried out for 10 wks and terminated at 17th wk and observations continued for another 7 wks afterwards.

The results were expressed as mean ± SEM. Differences between mean values of multiple or two groups were analyzed either by ANOVA or a Student's *t* test. Significance was accepted at $P<0.05$.

Results

Fig. 1 shows that BW increased during our experimental duration from 100 gm to 326 gm in M (solid circle), and 83 gm to 204 gm in F (solid triangle), respectively. At the beginning (7wk-old), M was already 17% ($P<0.001$) heavier than F; the difference in BW increased with aging and reached 60% ($P<0.001$) at 24th week. This 3-fold increase of BW demands the adjustment of L-NAME concentration in drinking water (see Methods). Treatment of L-NAME did not alter BW significantly in M (compared with M*, open circles). However, F gained less weight after 6-wks of L-NAME treatment (F*, open triangle), and actually loose weight at age of 16wk. F* had significantly higher BW than F during the period of 13 to 19wk of age as shown in the lower curves of Fig. 1. Thus the L-NAME treatment was terminated at 17th wk and afterward BW of F* recovered (showing net gain at 18th wk) and became similar to that of F during 20-24th wk. L-NAME treatment in general reduced food intake of WKYs; for example, at 16th wk, food intake for M and M* were 18.1 ± 0.5 and 16.3 ± 1.0 gm/wk, respectively; for F and F*, 15.2 ± 0.2 and 10.4 ± 1.1 gm/wk, ($P<0.001$) respectively.

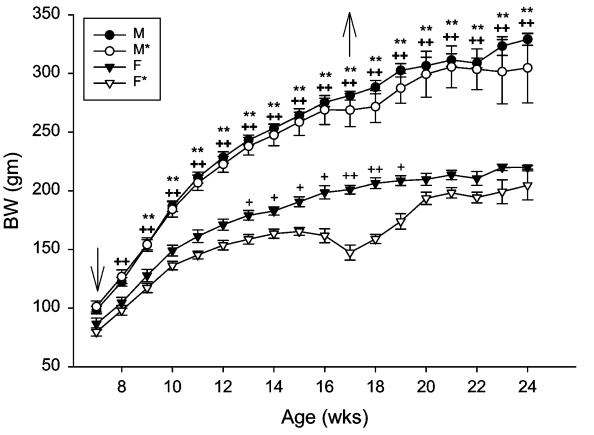

Fig. 1. Effect of L-NAME on BW of rats between age of 6-24 wk. M and F: control male and females (solid symbols); M* and F*: L-NAME-treated males and females (open symbols), respectively. M vs. F: * (light) ($P<0.05$), ** (light) ($P<0.001$); M vs. M*: * (bold) ($P<0.05$), ** (bold) ($P<0.001$); F vs. F*: + (light) ($P<0.05$), ++ (light) ($P<0.001$); M* vs. F*: + (bold) ($P<0.05$); ++ (bold) ($P<0.001$). ↓ and ↑ indicate the time points of administration and withdrawal of L-NAME, respectively.

These decreases of food intake in L-NAME-treated rats likely could account for the observed BW decrease (Fig. 1).

After L-NAME administration (↓ in Fig. 2) MAP started to increase and reached significant levels ($P<0.001$) for both genders at the 10th wk (Fig. 2). Furthermore, F* increased more and became significantly higher than M* ($P<0.001$). This gender difference of MAP elevation persisted for 4 wks more (10-14th wk) and disappeared afterwards (15-17th wk). L-NAME was thus withdrawn from the treatment rats at 17th wk (↑). We found that MAP dropped immediately in both genders and that F* decreased less than that of M* and thus resulted in a significant difference between M* and F* (Fig. 2) during 18-22th wk. There was no difference at 23-24th wk. Also, the elevation of MAP remained in the L-NAME-treated rats (M* and F*) even after 7 wks of L-NAME withdrawal. The pattern for SBP changes induced by L-NAME was similar to that of MAP except that gender difference (M>F) occurred earlier (8-12th wk) (data not shown).

In addition, we also determined HR and the data are shown in Fig. 3. There was a trend of decreasing HR as age increased and a significantly higher HR for F than M in control rats (solid symbols). In the L-NAME-treated WKYs (open symbols), a rapid decrease of HR occurred for both M* and F* (8th wk), consistent with previous reports (3-5). HR remained low for M* in the presence of L-NAME, however, it gradually increased for F* so that HR of F* was also significantly higher ($P<0.001$) than M* during the

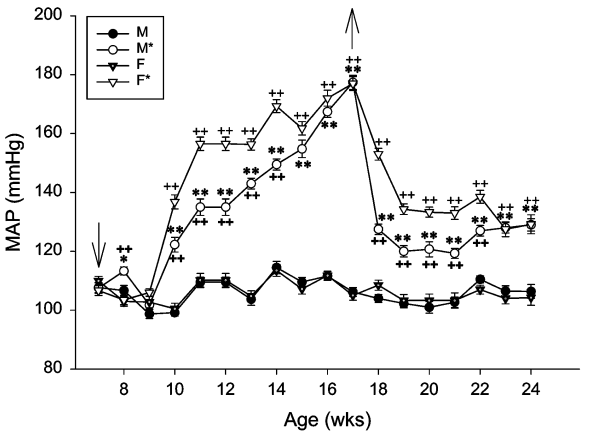

Fig. 2. Effect of L-NAME on MAP. Symbols are defined as that in Fig. 1 legend.

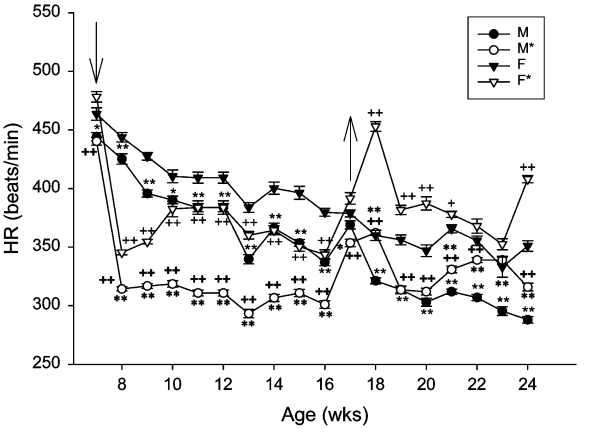

Fig. 3. Effect of L-NAME on HR. Symbols are identical to that given in Fig 1 legend.

whole experimental period (7-24th wk). Following L-NAME withdrawal, a rebound of HR occurred for both M* and F* (16-18th wk) and it gradually approached the HR of their respective counterparts M and F.

Discussion

The major findings of the present study are: 1. MAP increased significantly following L-NAME treatment (3-6) in both genders (M* and F*, Fig. 2) whereas female rats (F*) exhibited a more rapid and pronounced development of hypertension than M* for up to 5 wks. 2. Following withdrawal of L-NAME, partial reversal of the elevation of MAP took place in both genders whereas F* exhibited a slower and lesser decrease than M* for 5 wks, MAP of F* and M* eventually became identical but was still higher than control rats (Fig. 2). 3. HR decreased following L-NAME administration (4,5) in both genders and that the magnitude of the changes were significantly smaller for F* than that of M* (Fig. 3). 4. A rebound of HR occurred after L-NAME withdrawal and HR of F* became higher than F (Fig. 3). The reason for the different time course in changes of BP and HR (Fig. 2 vs. Fig.3) is probably due to the rapid effects on autonomic nerve activities. For example, NO is known to act centrally to enhance HR (17,18) in an acute manner and thus action of L-NAME could be expected to be in the opposite direction (17); furthermore, L-NAME also elevates cardiac parasympathetic tone (19). Taken together, although there was no gender difference at the highest level of MAP reached, L-NAME-treated females (F*) showed both aggravated response of BP elevation than males (M*) upon L-NAME treatment and attenuated response of BP lowering after L-NAME withdrawal for significant durations. Hence, for the first time, as far as we know, gender-dependent responses to L-NAME is demonstrated.

L-NAME induces increase of MAP and decrease of HR not only in rats (3-6) but also in primates (7). However, all these were performed only in male animals. Because females in general have a lower MAP than males (10), especially in hypertensive condition (12), it is worthwhile to examine the development of hypertension in females *visa-a-vis* males under L-NAME treatment.

The mechanisms for the apparent gender-dependent BP response are not clear. However, the critical role of NOS in the regulation of BP (11-15) provides a connection between L-NAME and gender difference. In a study where both duration (5 months) and dose (5 mg/kg/d) of L-NAME administration were similar to the current study, it was found that L-NAME caused significant increase of SBP and a significant negative correlation with the decrease of NO_3^- and cGMP in urinary excretion in male Wistar rats (8); indicating that the L-NAME-induced sustained hypertension is due to the inhibition of NO production (9). Although there was no difference in plasma cGMP, Arnal et al. (9) also found that L-NAME (10-100 mg/kg/d) treatment (4 wks) caused a time-and dose-dependent increase in SBP and decreased aortic cGMP by 10-fold in male Wistar rats. These evidence indicate that a major chronic action of L-NAME is to inhibit NO/cGMP pathway and thus to cause hypertension, possibly via the reduction of endothelium-dependent relaxation (8). In light of these evidence, our findings that female rats exhibited an augmented respone to L-NAME could be accounted for by assuming that females had higher NO level to begain with. Therefore, due to the action of estrogen on endothelial NO synthase (11,14,15), female rats depend on a higher NO/cGMP activity for their BP regulation and thus are more affected by L-NAME

during both the elevation phase of MAP upon L-NAME treatment and the recession phase of MAP after L-NAME withdrawal (Fig. 2).

Other connections between L-NAME administration and our observed gender-dependent response of BP are not excluded. These may include NO-independent gender difference of vascular function (20), other regulatory systems of BP that are affected by estrogen (21), and endogenous NOS inhibitor (22). These possibilities await further investigations to establish the precise mechanisun (s) of the observed intensified responses to NOS inhibitor in females.

Acknowledgments

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