

Influence of testosterone on mean arterial pressure: A physiological study in male and female normotensive WKY and hypertensive SHR rats

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Introduction: Testosterone plays an important role in the blood pressure regulation. However, information with regard to the effect of this hormone on blood pressure in normotensive and hypertensive conditions is limited. Therefore, in this study, the relationship between plasma testosterone level and mean arterial pressure (MAP) was investigated under these conditions. *Methods:* Normotensive Wistar-Kyoto (WKY) and hypertensive Spontaneous Hypertensive (SHR) male and female rats were gonadectomized with female rats treated with testosterone. Estrous cycle stages of intact female rats of both strains were identified by vaginal smear. Pressure in the carotid artery of anesthetized rats was measured via direct cannulation technique. The blood was withdrawn for plasma testosterone level measurement by enzyme-linked immunosorbent assay. *Results:* Treatment of ovariectomized female WKY and SHR rats with testosterone for 6-week duration has resulted in MAP to increase ($P < 0.05$). In male WKY and SHR rats, MAP and plasma testosterone levels decreased by orchidectomy ($P < 0.05$). No significant differences in MAP and plasma testosterone levels were observed in intact female WKY and SHR rats between stages of the estrous cycle. *Conclusions:* The effects seen in testosterone-treated ovariectomized female rats and in orchidectomized male rats suggested that testosterone could play an important role in causing the blood pressure to increase.

Keywords: testosterone, gonadectomy, blood pressure, rats, WKY, SHR

Introduction

The relationship between sex steroids and blood pressure has long been observed (22, 48). Gender difference in the blood pressure regulation has long been documented in which men have higher blood pressure as compared with age-matched women before menopause (10, 28, 42). Studies have shown that testosterone plays a role underlying the gender difference in blood pressure regulation. In men, there was an inverse relationship between blood pressure and plasma testosterone levels (16, 24, 47). Higher plasma testosterone level is associated with increased blood pressure in males, whereas in females, higher plasma testosterone level has been associated with increased arterial stiffness (16, 45). In addition, there is increased risk of hypertension in women possessing high plasma testosterone levels as in polycystic ovary disease (3, 12).

The effects of testosterone on blood pressure regulation have been documented in animals. In hypertensive rats, the levels of testosterone correlate with the mean arterial pressure (MAP) (10, 15, 23, 27, 37). However, in some studies, testosterone was found to

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have no effect on the blood pressure (8, 17). The effects of sex-steroid hormones on blood pressure in females are far more complex and less well understood as compared with the effects of these hormones on blood pressure in males. Female blood pressure was found to be influenced by estrogen (9, 18, 46).

To date, there have been limited findings with regard to the effect of testosterone on blood pressure regulation in females. The levels of this hormone were found to fluctuate throughout female reproductive cycle, which suggested that testosterone could influence the blood pressure as there is a mild blood pressure fluctuation observed throughout the cycle (26, 39, 40). In this study, the effects of testosterone on blood pressure regulation in females were investigated. In addition, the effects of removal of endogenous testosterone on blood pressure regulation in males were also investigated. Furthermore, influence of testosterone on blood pressure throughout the female reproductive cycle was also identified.

Materials and Methods

Animals

Eight-week-old male and female Wistar-Kyoto (WKY) and Spontaneous Hypertensive (SHR) rats were maintained in a well-equipped animal facility with a constant 12:12 h light:dark cycle. All animals had free access to a standard rodent chow diet (Teklad Diet, Rosscoff, Germany) and tap water *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC), University of Malaya with ethics number: 2014-05-07/physio/R/NS.

Experimental designs

In this study, three different experiments were designed and performed independently as follows:

Experiment 1. Male and female WKY and SHR rats were divided into four groups, each group contains five rats. Sham operations, orchidectomies, and ovariectomies were performed at 8 weeks of age in a sterile environment. At 16 weeks of age, animals were subjected for subsequent procedures. Experimental groups were designed as follows:

- Group 1:* Sham-operated, intact male WKY and SHR rats
- Group 2:* Orchidectomized (ORX), male WKY and SHR rats
- Group 3:* Sham-operated, intact female WKY and SHR rats
- Group 4:* Ovariectomized (OVX), female WKY and SHR rats

Experiment 2. Female WKY and SHR rats were used in this experiment. All animals were subjected for vaginal smear examination at 16 weeks of age. 0.9% (w/v) NaCl was flushed into the vagina and the fluid was then retrieved. Estrous cycle stage was determined based on the cells found in the vaginal flushing (unstained). The criteria for estrous stages identification were as described by Marcondes et al. (29). All rats were grouped in accordance with the estrous cycle stages, with five animals per group. Four experimental groups per strain were designed as follows:

- Group 1:* Proestrus stage (Ps)
- Group 2:* Estrus stage (Es)
- Group 3:* Metestrus stage (Ms)
- Group 4:* Diestrus stage (Ds)

Experiment 3. Male and female WKY and SHR rats were divided into four groups, each group contains five animals. Sham operations, orchidectomies, and ovariectomies were performed at 8 weeks of age. All surgeries were performed under sterile conditions. A group of ovariectomized female rats were implanted with testosterone 2 weeks after recovery for 6 weeks. For chronic testosterone administration, a 19-mm-long silastic tubing (Dow Corning Corporation, Auburn, MI; 0.062 in. ID; 9.125 in. OD) containing 10 mg of testosterone propionate (Sigma-Aldrich, St. Louis, MO, USA) was implanted subcutaneously at the back of the rat shoulder, following the method as described by Reckelhoff et al. (36). Non-testosterone-treated rats were implanted with empty silastic tubing. The tubings were replaced every 3 weeks. When animals achieved 16 weeks of age, they were subjected for the procedures. Four experimental groups per strain were designed as follows:

Group 1: Sham-operated, intact male WKY and SHR rats

Group 2: Orchidectomized (ORX) male WKY and SHR rats

Group 3: Ovariectomized (OVX) female WKY and SHR rats

Group 4: Ovariectomized (OVX) and testosterone-treated (T) female WKY and SHR rats

In vivo blood pressure measurement

Rats were anesthetized at 16 weeks of age by pentobarbital sodium anesthesia (60 mg/kg; Sigma-Aldrich), which was injected intraperitoneally and the dose was maintained throughout the period of anesthesia. Cannulation of the carotid artery follows the method as described by Parasuraman and Raveendran (34). In brief, a small incision was made in the trachea to insert a tracheostomy tube and a cannula pre-filled with heparinized normal saline (0.5 IU/ml) was then inserted into the artery. The cannula was connected to a PowerLab Data acquisition system (ADInstruments, Australia), which then directly monitors the blood pressure. Blood pressure was recorded after the rats were stabilized at least for 10 min. Data were analyzed by LabChart version 6.0 where the average systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained. MAP was then calculated.

Measurement of plasma testosterone level

At the end of cannulation, blood was collected from the artery and placed into a chilled heparinized tubes. Blood plasma was obtained by centrifuging at $3,000 \times g$ for 20 min at 4 °C. Plasma testosterone levels were measured using enzyme-linked immunosorbent assay kit (Enzo Life Sciences, Farmingdale, NY, USA) (Cat No. ADI-900-065), following the manufacturer's guidelines. Each assay was performed in triplicate with the lowest sensitivity limit of 5.67 pg/ml.

Statistical analysis

All data were presented as mean \pm standard error of mean (SEM). All statistical analyses were performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). Statistical differences between groups were evaluated using independent unpaired Student's *t*-test. Comparisons between more than two groups were performed using one-way analysis of variance with Tukey's *post-hoc* test. Differences were considered as statistically significant at *P* level < 0.05.

Results

Effects of gonadectomy on MAP and plasma testosterone level in males and female rats

In sham-operated male rats, MAP was higher than sham-operated female rats for both rat strains ($P < 0.001$) (Fig. 1a). Orchidectomy caused a decrease in MAP in male rats ($P < 0.01$ for WKY rats and $P < 0.001$ for SHR rats). In female rats, ovariectomies also caused a decrease in MAP ($P < 0.05$ for WKY rats and $P < 0.01$ for SHR rats).

The highest plasma testosterone levels were observed in sham-operated male SHR rats (Fig. 1b). Orchidectomy decreased the plasma testosterone level in both male WKY ($P < 0.001$) and SHR ($P < 0.05$) rats. Similarly, ovariectomy also decreased the plasma testosterone level in female rats of both strains ($P < 0.05$).

Changes in MAP and plasma testosterone levels at different stages of the rat estrous cycle

No differences in MAP were reported across the estrous cycle stages for any of the WKY and SHR female rats (Fig. 2a). Similarly, plasma testosterone levels were not significantly different between stages of the estrous cycle in any of the rat strains (Fig. 2b).

Effects of testosterone on MAP and plasma testosterone levels in female rats

In ovariectomized female rats, 6-week treatment with testosterone caused the MAP to increase ($P < 0.05$ for WKY rats and $P < 0.01$ for SHR rats) (Fig. 3a). In concordant, plasma

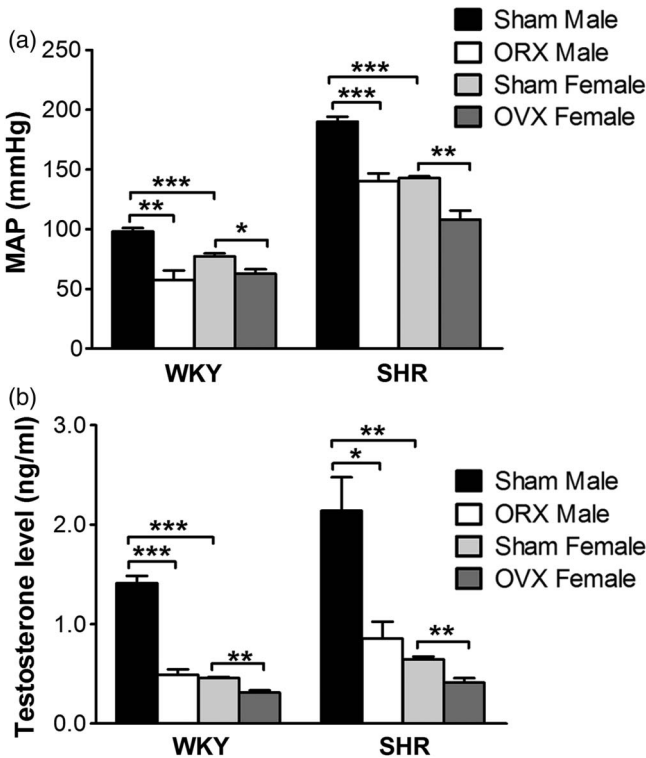


Fig. 1. (a) MAP of 16-week-old WKY and SHR male and female rats as measured by cannulation of the carotid artery. Sham operation and gonadectomies were carried out at 8 weeks of age. (b) Plasma testosterone levels were measured using the blood collected from the carotid artery at the end of the experiment. Data were presented as mean \pm SEM; $n = 5/\text{group}$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Sham: sham-operated; ORX: orchidectomized; OVX: ovariectomized

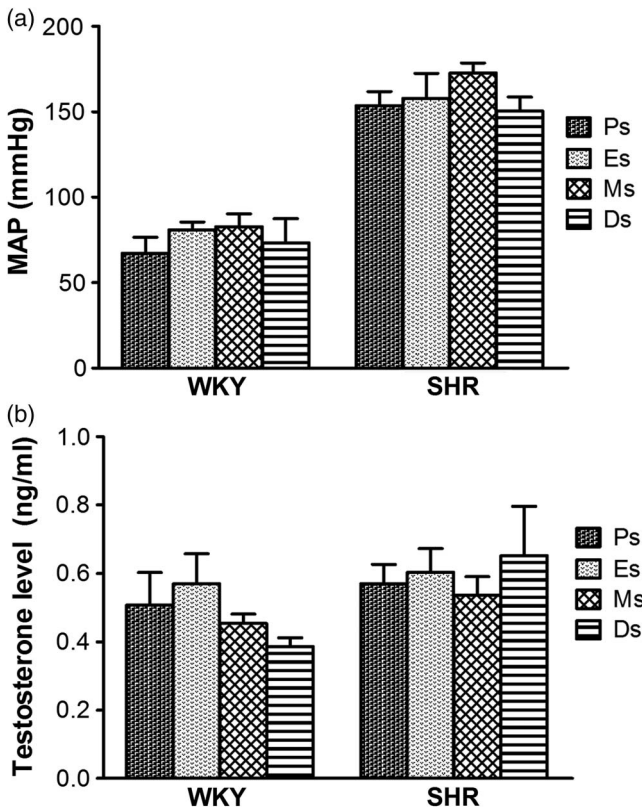


Fig. 2. (a) MAP of 16-week-old naive female WKY and SHR rats as measured by cannulation of the carotid artery. The stages of estrous cycle were determined by vaginal smears. (b) Plasma testosterone levels were measured using the blood collected from the carotid artery at the end of the experiment. Data were presented as mean \pm SEM; $n = 5-8$ /group. Ps: proestrus; Es: estrus; Ms: metestrus; Ds: diestrus

testosterone levels in female rats receiving testosterone treatment markedly increased ($P < 0.05$) (Fig. 3b).

Discussion

Sex steroids are known to play an important role in the blood pressure regulation in males and females (19, 21, 28). In this study, a positive correlation between plasma testosterone level and MAP has been identified. MAP was found to be higher in intact male WKY and SHR rats as compared with intact female WKY and SHR rats and these findings were consistent with the reports by others (4, 7, 30, 31, 35). In this study, gonadectomies performed prior to pubertal age, that is, 8 weeks were found to decrease the MAP in both male and female normotensive WKY and SHR rats. These findings were also consistent with the report by Masubuchi et al. (31) who showed that the blood pressure in adult male and female rats could be affected by gonadectomies. In male rats, it was found that orchidectomy caused a decrease in the levels of endogenous testosterone, whereas in female rats, ovariectomy caused a decrease in endogenous testosterone levels.

Changes in plasma testosterone levels were found to positively correlate with MAP, as seen in male and female intact and gonadectomized rats and in gonadectomized female rats receiving exogenous testosterone treatment. The increase in MAP in ovariectomized female

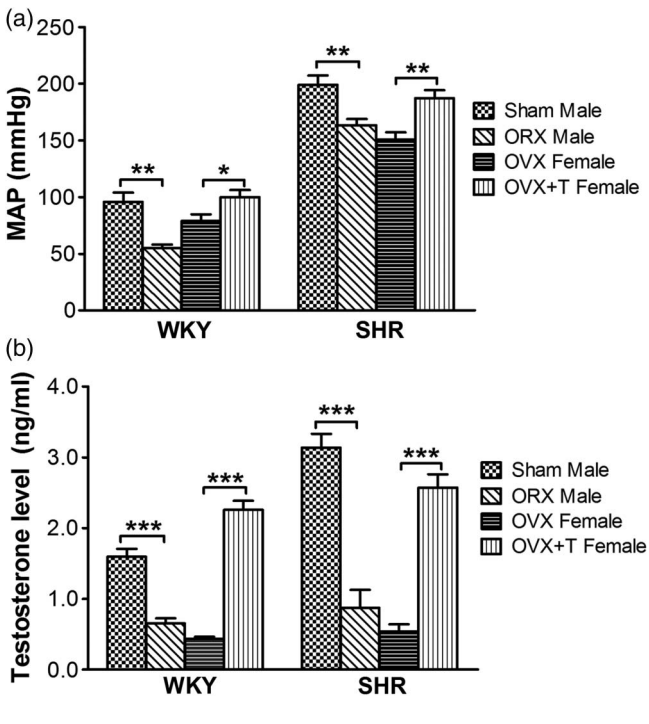


Fig. 3. (a) MAP of 16-week-old WKY and SHR male and female rats as measured by cannulation of the carotid artery. Sham operation and gonadectomies were performed at 8 weeks of age. At the age of 10 weeks, female rats were treated with testosterone implants for 6 weeks. (b) Plasma testosterone levels were measured using the blood collected from the carotid artery at the end of the experiment. Data were presented as mean \pm SEM; $n = 5$ /group; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Sham: sham-operated; ORX: orchidectomized; OVX: ovariectomized; T: testosterone

rats of both strains following exogenous testosterone treatment strengthened the argument that testosterone increases the blood pressure as this model eliminates the effect of male sex chromosome, which has been implicated in causing higher blood pressure in males compared with females (13, 14, 32). Our findings were supported by the results of Reckelhoff et al. (36) who demonstrated an increase in MAP in testosterone-treated female SHR rats. In this study, increase in MAP was also seen in testosterone-treated normotensive WKY rats, indicating that testosterone-induced increase in blood pressure could also happen in the normotensive condition. The findings in male WKY and SHR rats in which orchidectomy had also caused a decrease in blood pressure have further strengthened the view that testosterone is involved in increasing the blood pressure, a finding similar to that found by others (23).

There were conflicting reports with regard to the effect of ovariectomy on MAP (9, 35, 42). Ovariectomy has been reported not to cause changes in blood pressure of wild-type mice. In another study using Sprague-Dawley (SD) rats at 10–12 weeks of age, no difference in the MAP between intact and ovariectomized female rats was found (49). In another study, Crofton and Share (6) have shown that in female SD rats, gonadectomy did not cause significant changes in blood pressure when compared with intact rats. Their study further showed that gonadectomized female rats were more sensitive toward deoxycorticosterone-salt induced increase in the blood pressure. Ovariectomy performed in 6-week-old female Dahl salt-sensitive rats which were given low-sodium diet did not cause any significant changes in blood pressure at 12 weeks (3 months), however at 16 weeks (4 months), the SBP in these rats was significantly higher than in intact rats, and these differences persisted up to the age of 84 weeks (12 months) (20).

In contrast, our findings and several other findings indicated that ovariectomy reduced the blood pressure in female rats. Stachowiak et al. (44) reported that 28 days after ovariectomy, the blood pressure in female rats markedly decreased which was associated with a marked atrophy of the adrenal cortices. A recent study also indicated that there was a trend of a reduction of the blood pressure taken at 24 weeks of age in female SD rats where ovariectomy was performed at 7 weeks of age (5). A study conducted in 12-week-old Wistar rats indicated that there was a transient decrease in MAP measured using a tail cuff, 2 weeks after ovariectomy which subsequently returned to pre-ovariectomized values (25). In this study, we measured the blood pressure in anesthetized female rats via carotid artery cannulation which showed similar results.

We hypothesized that the decrease in MAP following ovariectomy in female WKY and SHR rats may be, at least partly, mediated by suppression of adrenal secretory activity, due to the lack of circulating estrogens, which are known to stimulate the hypophyseal adrenocorticotropic hormone release. Estrogen has been reported to be able to increase the rennin-angiotensin system activity (33, 41). Therefore, a decrease in estrogen levels following removal of endogenous source of estrogen via ovariectomy will result in the alleviation of estrogen-induced increase in renin-angiotensin-aldosterone system activity, contributing to the reduction in blood pressure.

This study has shown that there was no significant difference in MAP during the rats' estrous cycle. Previous studies have also demonstrated that blood pressure did not change throughout the estrous cycle in rodents (2, 11). Liu and Ely (27) also reported that the blood pressure in female SHR rats did not change throughout the estrous cycle, a finding consistent with ours. The fact that there were no changes in blood pressure observed throughout the estrous cycle indicated that fluctuation in endogenous sex-steroid levels throughout the cycle had no influence on the blood pressure. Despite the high estrogen levels at estrus and high progesterone levels at diestrus, these hormones seem not to have an influence on the blood pressure. On the other hand, plasma testosterone levels did not change significantly throughout the estrous cycle (40). Therefore, it can be concluded that in intact female rats, the blood pressure changes rather depend on other factors than the fluctuating levels of sex-steroid hormones.

This study has several limitations. The blood pressure measurements made in anesthetized rats might not reflect the changes in blood pressure of conscious animals. Future studies should be performed using a radio-telemetry device that can provide a real-time blood pressure monitoring in conscious, freely moving animals (1). In conclusion, this study may have implications in the understanding of gender difference in blood pressure regulation. Testosterone might be implicated in causing higher incidence of hypertension in men, and in women after menopause (38). The elevated MAP in post-menopausal women could partly be related to the elevated levels of plasma testosterone, in addition to the falling levels of plasma estrogen (43, 50).

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Conflict of interest

The authors declare no conflict of interest.

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