

The effects of androgen on sodium excretion and the renin-angiotensin system in high salt-induced hypertensive male rats.

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Hypertension is a common cardiovascular disease worldwide. Several observations suggest that gender differences exist in the development of hypertension. Generally, males have a higher blood pressure than females in both humans and animals, suggesting that androgens participate in the development of hypertension. It has been evidenced that androgen has a potential role in controlling blood pressure in both humans and rats. High salt diet is an important risk factor contributing to hypertension. Caplea¹ observed that androgen receptor blockade reduced sodium-induced blood pressure rise in male rats, suggesting that androgens participate in blood pressure elevation in sodium-induced hypertension, but there is little information concerning mechanisms involved in the process. There is increasing evidence to support that androgens influence renal sodium metabolism and the renin-angiotensin system, which play a key role in the development of hypertension.^{2,3} This study aimed to explore the role of androgens in the development of hypertension in high salt intake male rats, and the associated mechanism including urine-sodium excretion, or the renin-angiotensin system.

In high salt dietary male rats, we measured the variation of blood pressure (BP), urine Na excretion, plasma AngII and renin-activity (PRA) in different testosterone contents or androgen receptor (AR) blockade *in vivo*. Sixty male Wistar rats weighing 200±20 g, aged 8 weeks (from the Chinese Academy of Sciences) were used in this study. The rats were housed on a 12-hour day/night cycle room maintained at a temperature of 20-26°C, and a humidity of 40-70%. A high salt (8%) intake diet (from the Chinese Academy of Sciences) and tap water were available *ad libitum*. The procedure was carried out in Animal Experimental Center in Shanghai Sixth People's Hospital, during the period of July 2007 to September 2008, and was approved by the ethical committees of our hospital. Rats were randomly divided into 5 groups, 12 rats per group: sham operation group (Sham), castration group (Cas), testosterone replacement after castration group (TC), androgen receptor blocker flutamide treatment to castration group (FC), and flutamide treatment to TC group (FTC). Sham group had sham operation and the other 48 underwent orchidectomy (ORC) after

2 weeks adaptation period. Each rat was anesthetized intraperitoneally with 2% ketamine hydrochloride 0.2 ml in a clean environment, and had their bilateral testes exposed. The 48 rats then underwent bilateral ORC, and the other 12 rats had sham operation. Group TC and FTC that were subjected to testosterone replacement at the time of castration were given testosterone undecenoate (TU 4 mg/kg/2weeks (Xianju Pharmaceutical Co Ltd, Zhejiang, China) by intramuscular injection, while the others received the same volume of tea seed oil. Meanwhile, group FC and FTC were intragastrically administered with flutamide (83 mg/kg, daily for 8 weeks (Fudan-Fuhua Pharmaceutical Co Ltd, Shanghai, China) the other rats were fed with the same saline. Following which, serum was obtained from all rats for measurement of testosterone. All rats were fed with 8% NaCl pellet feed for 8 weeks, and each group had equivalent basal diets. Blood pressure was recorded at the start of the experiment, at the end of 4 and 8 weeks. The BP was measured by the tail-cuff method. The rat was fixed, and the tail was heated for 30 min before measurement, and then systolic BP was recorded by rat arteria caudalis blood pressure determinator (RBP-1, China-Japan Friendship Clinical Medicine Research Institute, Beijing) 3 times for each rat to obtain the average value. At the end of the study, all the rats were anesthetized again and had their hearts exposed fully. Blood samples were obtained from the hearts and maintained in anticoagulation tubes with enzyme inhibitor or common tubes prepared beforehand. All tubes were sealed, and samples well mixed, and placed at a temperature of 4°C for 1-2 hours. Samples were centrifuged at 2500 g at a temperature of 4°C for 7 minutes. The supernatant was collected, which was peripheral blood serum or plasma, and then stored at a temperature of -80°C. The week before the rats were sacrificed, they were placed in metabolism cages with one rat in one cage. Urine was collected for 3 days continuously for measurement of sodium concentration. Twenty-four hour urine-sodium excretion was the value of the equation: sodium concentration x the average urine volume of 3 days. Testosterone, PRA, and angiotensin II (AngII) content were assayed by radioimmunoassay. After thawing in cold water, and strictly following the process in the manual of the kits (Northern Institute of Biotechnology, Beijing, China) the examination of serum or plasma samples were carried out in γ -radioimmunoassay counting instruments (GC-120, Zhongjia Photoelectric Instrument Co, China Science and Technology University, Hefei, China).

Data are presented as mean±SD, comparisons of experimental parameters among groups were performed by one-way ANOVA. Equal variances assumed, the

SNK test was used while equal variances not assumed, Dunnett's T3 was used. A $p < 0.05$ was considered statistically significant. Statistical Package for Social Sciences version 13.0 (SPSS Inc., Illinois, USA) statistical software was used for data analysis and processing.

Blood pressure. There were no differences in the baseline values among the 5 groups. However, manipulation of androgen had a significant effect on systolic BP. In the same conditions of high salt intake, the Sham group had a significant rise in BP at the end of 8 weeks, while the Cas group or FC had no significant rise at the end of 8 weeks. This effect of castration was largely reversed when the Cas group rats were subjected to testosterone replacement. Meanwhile, FTC mainly retarded the effect of testosterone on BP rise.

Testosterone content. Group Cas or FC had a significant decrease of serum testosterone content compared with the Sham group, while group TC had a similar testosterone content as sham did. Group FTC dramatically raised the testosterone content by 9 times.

Twenty-four hour urine sodium excretion. The average levels of 24-hour urine-sodium excretion are shown in

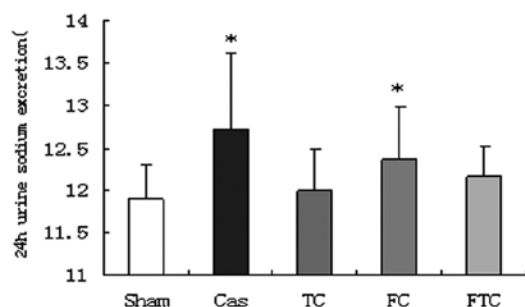


Figure 1 - The average level of 24h urine sodium excretion in each groups. * $p < 0.05$, Cas, FC versus Sham, Cas and FC had a higher urine-sodium excretion compared with Sham or TC group, FTC also increased the excretion although without significant statistical difference. Sham - sham operation, Cas - castration, TC - testosterone replacement after castration, FC - flutamide treatment to castration, FTC - flutamide treatment to TC.

Figure 1. The Cas or FC groups had a significant increase of urine-sodium excretion versus the sham. While the TC group reserved the effect of castration on sodium excretion. The FTC group had an increased 24-hour urine sodium excretion by 17% compared to Sham, although there was no statistical significance observed (Figure 1).

The PRA, and plasma AngII content. Group Cas or FC had a significant lower PRA or plasma AngII than the sham group. Group TC reversed the effect of castration on the results, and had a similar PRA or plasma AngII content to that of the sham group. While group FTC had a similar effect on PRA and plasma AngII content as castration did, that is to say, flutamide treatment largely reduced PRA and plasma AngII content compared to the sham group. All the data of the results in this study are shown in Table 1.

Previous studies showed that AR blockade ameliorated sodium-induced hypertension in rats.¹ In the present study, we also found that in the high salt-intake male rats, castration retarded but testosterone replacement propelled blood pressure elevation, while androgen receptor blockade had partly the similar effects as castration did. The results suggest that androgen binding with AR contributes to the mechanism of sodium-induced hypertension development in male rats. The present study showed that testosterone affected urine-sodium excretion, which is an important factor of BP variation. From the results, we could see the trends of 24-hour urine- sodium excretion. Castration or AR blockade increased the excretion compared with the control, and then testosterone replacement decreased it. The mechanisms by which androgen regulates sodium excretion are far from elucidated. Most actions of androgen are mediated by AR, and recent research has found that AR is not only expressed in the proximal tubule, but also in the cortical collecting duct (CCD),⁴ suggesting that androgens influence electrolyte excretion via AR. Toot et al⁵ observed a significant increase in sodium excretion in both SHR/y and WKY male rats with AR blockade. In the present

Table 1 - The result of detective index in each group.

Groups	T (ng/ml)	Base-BP (mm Hg)	8w-BP (mm Hg)	Na-extract (mmol)	PRA (ng/ml.h)	AngII (pg/ml)
Sham	0.28 ± 0.02	116.0 ± 48	136.1 ± 4.3*	11.90 ± 0.40	5.98 ± 0.72	508.91 ± 52.57
Cas	0.05 ± 0.01†	116.9 ± 5.7	122.8 ± 5.7†	11.72 ± 0.90‡	4.92 ± 0.55‡	393.77 ± 58.06†
TC	0.35 ± 0.06	117.8 ± 4.6	133.3 ± 3.7*	12.00 ± 0.72	5.81 ± 0.72	503.40 ± 38.37
FC	0.08 ± 0.01†	117.0 ± 5.5	122.0 ± 5.0†	12.36 ± 0.63‡	5.00 ± 0.52‡	406.75 ± 24.49†
FTC	3.05 ± 0.37†	115.7 ± 5.3	127.7 ± 5.5†	12.16 ± 0.37	5.12 ± 0.36‡	428.92 ± 22.05†

*Compared with baseline-blood pressure (BP), $p < 0.01$, † compared with Sham, $p < 0.01$, ‡ compared with Sham, $p < 0.05$. T - testosterone content, 8w-BP - 8 weeks blood pressure, Na-excr - 24 hour urine-sodium excretion, PRA - plasm renin activity, AngII - plasm angiotensin II content, Cas - castration, TC - testosterone replacement after castration, FC - flutamine treatment to castration, FTC - flutamine treatment to TC

study, AR blockade flutamide treatment also increased urine-sodium excretion, but did not reach significant difference as castration did. The results suggested that the AR pathway, by which testosterone regulates sodium metabolism, may not be the main one. Recently, Boulkroun et al⁴ showed that androgen regulated the expression of N-myc downstream-regulated gene 2 (NDRG2), which is an early aldosterone-induced gene located specifically in the CCD. Androgen directly regulates the renal electrolyte transport channel gene expression, which maybe another pathway of androgen affecting urine-sodium excretion. In addition, renal renin-angiotensin components may contribute to the change of urine metabolism. The present study showed that testosterone increased plasma AngII, and reduced urine-sodium excretion. Plasma AngII may activate aldosterone, which increases urine-sodium, and water resorption. Consistent with this study, Quigley² observed that enalaprilat or losartan decreased the proximal tubule resorption rate in dihydrotestosterone (DHT)-treated rats to a greater degree than in control rats. As described above, the mechanisms by which androgen regulates urine-sodium metabolism are complicated. In the next study, we should further study the associated mechanisms. In addition, androgen positively regulates the renin-angiotensin system (RAS). Reckelhoff et al³ observed that angiotensin-converting enzyme inhibitor (ACEI) reduced the testosterone-induced BP elevation in SHR rats. Androgens regulate RAS through the AR pathway.^{1,3} The present study showed that testosterone binding with AR promoted PRA and plasma AngII content in high salt intake male rats, AR blockade reduced the PRA and circular AngII as castration did, our data were consistent with previous studies.

The present study demonstrated that AR blockade largely retarded testosterone's efficiency. Flutamide treatment had a similar effect to castration, but the efficacy was gentler. The results showed that androgen was

not mediated by AR absolutely, but partly. Testosterone would convert to estradiol, which retarded the efficacy of androgen *in vivo*, and testosterone regulated Ca²⁺ or K⁺ channel activity, or expression of vascular smooth muscle via the non classic-AR pathway. All these may explain the result that AR blockade was partly, but not fully efficient as castration.

In conclusion, androgens participate in sodium-induced blood pressure elevation in male rats through ARs associated with renal natrium metabolism and the renin-angiotensin system. However, the clear mechanism by which androgen regulated BP is not clearly understood and warrants further investigation.

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