Inhibition of Aldosterone Production by Testosterone in Male Rats

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In vivo and in vitro experiments were designed to assess the effect of testosterone on aldosterone secretion in male rats. Orchidectomized rats were injected subcutaneously with oil or testosterone propionate ([TP] 2 mg/kg) for 7 days. Intact rats were injected with oil only. The results indicate that the plasma aldosterone level was higher in orchidectomized versus intact and TP-replaced rats. In the in vitro study, testosterone caused a marked decrease of aldosterone secretion by zona glomerulosa (ZG) cells, but failed to alter the accumulation of intracellular adenosine 3′,5′-cyclic monophosphate (cAMP). Testosterone significantly decreased the corticotropin (ACTH)-stimulated production of aldosterone and accumulation of cAMP in rat ZG cells. The conversion of corticosterone to aldosterone and of 25-OH-cholesterol to pregnenolone, as well as angiotensin II (ANG II)-stimulated production of aldosterone, were decreased by testosterone. These results suggest that testosterone inhibits the basal and ANG II- and ACTH-stimulated release of aldosterone, via inhibition of aldosterone synthase activity and cytochrome P-450 side-chain cleavage (P450scc) activity, and ACTH-stimulated cAMP accumulation in rat ZG cells.

ALDOSTERONE is the main mineralocorticoid produced in the zona glomerulosa (ZG) of the adrenal cortex in most mammalian species. Its major physiological role is sodium retention and potassium excretion in the kidney. The two limiting steps in the aldosterone biosynthetic pathway are, respectively, the early step (cytochrome P-450 side-chain cleavage [P450scc]) and the last step (18-hydroxylase-isomerase or aldosterone synthase).[1,2] Angiotensin II (ANG II) is an important regulator involved in the stimulation of aldosterone secretion.[3] In physiological conditions, corticotropin (ACTH) is responsible for maintaining the activity of enzymes in the early step and the increase in plasma aldosterone following acute stress.[4] In general, these stimulatory factors modify aldosterone secretion by changing the activity at one or both of the two limiting steps in the biosynthetic pathway.[3]

Sex differences in renin-angiotensin responsiveness[5] and hypothalamic-pituitary-adrenal activity[6,7] have been studied extensively, leading to the belief that the differences are attributable to the effects of sex hormones.[8] Vernikos et al[5] reported that compared with men, women had a lower systolic pressure and a higher plasma renin activity and aldosterone response to quiet standing for 2 hours. Long-term treatment with high-dose androgen for 24 days increased angiotensinogen mRNA in the kidney in the rat.[9] Wagner et al[10] demonstrated a significant androgen-dependent increase of renin gene expression in the kidney after 7 days of androgen treatment. In the rat, females react to stress with a greater increase in circulating ACTH and corticosterone compared with males.[7] It has been demonstrated that castration enhances and androgen replacement suppresses the ACTH and corticosterone response to a physical or psychological stressor.[11] Bingaman et al[12] have shown that castration increases hypothalamic corticotropin-releasing hormone (CRH) content and CRH-immunoreactive cell numbers in the paraventricular nucleus, and that CRH responses to castration can be reversed by administration of androgen.

Long-term androgen treatment of rats leads to the development of hypertension and cardiovascular disease.[13] The androgen effect on the cardiovascular system is believed to be mediated through the adrenal gland,[14] particularly by an action on the steroid 11β-hydroxylase enzyme system, including a decrease in 11β-hydroxylase activity and cytochrome P-450 11β mRNA.[15] Moreover, androgen receptors have been characterized in the rat adrenal gland.[16] Based on these observations, we hypothesize that testosterone is involved in the regulation of aldosterone release. Recent studies have demonstrated that gonadal steroid hormones have acute (nongenomic) effects on neural tissue,[17] Sertoli cells,[18] and vascular smooth muscle cells.[19] Whether testosterone exerts nongenomic effects on adrenal ZG cells is still unclear.

In the present study, we examined the effects of testosterone on aldosterone secretion both in vivo and in vitro. To determine the mechanisms of action of testosterone, we assessed the effects of testosterone on ANG II- or ACTH-induced aldosterone secretion and adenosine 3′,5′-cyclic monophosphate (cAMP) accumulation and steroidogenesis in rat ZG cells.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 300 to 350 g were housed in a temperature-controlled room (22° ± 1°C) with 14 hours of artificial illumination daily (6 AM to 8 PM) and given food and water ad libitum.

In Vivo Experiment: Effects of Orchidectomy and Testosterone Replacement on Plasma Aldosterone Concentration in Male Rats

Male rats were orchidectomized for 2 weeks before subcutaneous injection of sesame oil (Sigma, St Louis, MO) or testosterone propionate ([TP] 2 mg/kg; Fluka, Buchs, Switzerland) once daily for 7 days.

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Intact male rats received sesame oil only. Blood samples were collected for measurement of testosterone and aldosterone levels by radioimmunoassay (RIA). The plasma was separated by centrifugation at 10.000 × *g* for 1 minute, mixed with diethyl ether (10-fold vol), shaken for 30 minutes, centrifuged at 1,000 × *g* for 5 minutes, and quick-frozen in a mixture of acetone and dry ice. The organic phase was collected, dried, and reconstituted in borate buffer (pH 7.8) containing 1% bovine serum albumin (BSA) before measurement of aldosterone by RIA. Plasma levels of Na^+, K^+, and Ca^{2+} were determined by flame photometry (EFOX 5053; Eppendorf, Hamburg, Germany). To confirm the effects of orchidectomy and TP replacement, plasma testosterone levels were measured by RIA and the seminal vesicles were weighed after decapitation.

Preparation of ZG Cells

Male rats were decapitated in the morning. The adrenal glands were rapidly removed and stored in 0.9% (wt/vol) NaCl in an ice bath. The technique used for the preparation of ZG cells was modified from the method of Purdy et al.[20] Briefly, after removal of excess fat, the glands were separated into capsule (mainly ZG) and inner-zone (mainly zona fasciculata/reticularis) fractions. Capsules from five to eight adrenal glands were designated as one dispersion and were added to a polyethylene tube containing 1 mL Krebs-Ringer bicarbonate buffer with 3.6 mmol/L K^+, 11.1 mmol/L glucose, and 0.2% BSA (KRBGA medium) and 2 mg collagenase (Sigma). The tube was aerated with 95% O_2 and 5% CO_2 and incubated for 1 hour at 37°C in a shaker bath oscillating at 100 cycles per minute. Generally, at least six dispersions (n = 6) of ZG cells were included in each group. At the end of the incubation, the capsular tissues were mechanically dispersed into cells by repeated pipetting and finally filtered through nylon mesh. After centrifugation at 200 × *g* for 10 minutes, the cells were washed with deionized water to disrupt the red blood cells, and the osmolarity was immediately restored with 10-fold Hank's balanced salt solution. Cell number and viability (>76%) were determined using a hemocytometer and the trypan blue–exclusion method. Cells were diluted to a concentration of 5×10^4 cells/mL and divided into the test tubes The cells were then preincubated with incubation medium for 1 hour at 37°C in a shaker bath (100 cycles per minute) aerated with 95% O_2 and 5% CO_2. The supernatant was decanted after centrifugation of the tubes at 200 × *g* for 10 minutes.

In Vitro Experiments

To determine the effect of testosterone on basal (unstimulated) or ACTH-stimulated aldosterone secretion, ZG cells (5×10^4 cells) were incubated with KRBGA medium (vehicle) and ACTH (10^{-9} mol/L) in the absence or presence of testosterone (10^{-9} to 10^{-7} mol/L) for 2 hours. To terminate the incubation, 0.2 mL ice-cold KRBGA medium was added. The medium was centrifuged at 200 × *g* and stored at −20°C until analysis for aldosterone by RIA. To study the effect of testosterone on cAMP accumulation, ZG cells were primed with 5×10^{-4} mol/L 3-isobutyl-1-methylxanthine ([IBMX] Sigma), a phosphodiesterase inhibitor, for 1 hour. ACTH (10^{-9} mol/L), testosterone (10^{-9} to 10^{-7} mol/L; Sigma), or ACTH plus testosterone (10^{-9} to 10^{-7} mol/L) in 0.3 mL KRBGA medium mixed with IBMX were added to the tubes and incubated with the ZG cells for 2 hours. At the end of incubation, the cells were mixed with 0.5 mL 65% ice-cold ethanol, homogenized by a polytron homogenizer (PT-3000; Kinematica, Lucerne, Switzerland), and centrifuged at 2.000 × *g* for 15 minutes. The supernatants were lyophilized in a vacuum concentrator (Speed Vac; Savant, Holbrook, NY) and reconstituted with assay buffer (0.05 mol/L acetate buffer with 0.01% sodium azide, pH 6 2) before measuring the concentration of cAMP by RIA.

To assess the effects of testosterone on ANG II-stimulated aldosterone secretion and the aldosterone synthase, ZG cells were incubated with KRBGA, testosterone (10^{-9} to 10^{-7} mol/L), ANG II (10^{-7} mol/L), or ANG II plus testosterone (10^{-8} or 10^{-7} mol/L) in the absence or presence of corticosterone (10^{-7} to 10^{-5} mol/L; a substrate of aldosterone synthase; Sigma) for 2 hours. After 2 hours of incubation, the supernatants were collected for determination of aldosterone by RIA To study further the effects of testosterone on the activities of P450scc, 3β-hydroxysteroid dehydrogenase (3β-HSD), 21-hydroxylase, and 11β-hydroxylase, the cells were incubated for 2 hours either with or without precursors (10^{-6} mol/L), including 25-OH-cholesterol (a membrane-permeable cholesterol, substrate of P450scc, Sigma), pregnenolone (substrate of 3β-HSD: Sigma), progesterone (substrate of 21-hydroxylase: Sigma), or deoxycorticosterone (substrate of 11β-hydroxylase; Sigma), in the absence or presence of testosterone (10^{-8} or 10^{-7} mol/L). At the end of the incubation, the medium was collected and the concentrations of pregnenolone, progesterone, corticosterone, and aldosterone were measured by RIA.

RIA of aldosterone. The aldosterone concentration in both plasma and medium samples was measured by RIA as described previously.[21] The antialdosterone antiserum (no. 088) was provided by US National Institutes of Health. The aldosterone RIA sensitivity was 4 pg per assay tube. The intraassay and interassay coefficients of variation were 5.5% (n = 5) and 7.6% (n = 5), respectively. The inhibition curves produced by ether-extracted rat plasma and the incubation medium of rat ZG cells were parallel to the curves produced by unlabeled aldosterone.

RIA of testosterone. The plasma testosterone concentration was determined by RIA as described elsewhere.[22] The testosterone RIA sensitivity was 2 pg per assay tube. The intraassay and interassay coefficients of variation were 4.1% (n = 6) and 4.7% (n = 10), respectively.

RIA of pregnenolone. The antipregnenolone antiserum was purchased from Biogenesis (Sandown, NH). Cross-reactivity was 67% with pregnen-36-ol-20-one sulfate; 19% with progesterone; and 3% or less with 17α-hydroxypregnenolone, cholesterol, 17α-OH-progesterone, 20α-di-OH-progesterone, cortisol, deoxycorticosterone, corticosterone, aldosterone, androstenedione, testosterone, dihydrotestosterone, etiocholanolone, estradiol, estrone, or estriol. For the RIA system, a known amount of unlabeled pregnenolone or an aliquot of rat ZG cell medium, adjusted to a total volume of 0.3 mL by a buffer solution (0.1% gelatin-phosphate-buffered saline [PBS], pH 7.5), were incubated with 0.1 mL pregnenolone antiserum (1:400) diluted with 0.1% gelatin-PBS and 0.1 mL [^{3}H]-pregnenolone (8,000 cpm; Amersham, Buckinghamshire, UK) at 4°C for 24 hours. Duplicated standard curves of pregnenolone were incubated in each assay. An adequate amount (0.2 mL) of dextran-coated charcoal (0.25%) was added and further incubated in an ice bath for 15 minutes. After incubation, the assay tubes were centrifuged at 1,500 × *g* for 40 minutes. The supernatant was mixed with 3 mL liquid scintillation fluid (Ready Safe, Beckman, Fullerton, CA) before radioactivity determination in an automatic beta counter (Wallac 1409; Pharmacia, Turku, Finland). The sensitivity of the pregnenolone RIA was 16 pg per assay tube. The inhibition curves produced by ZG cell medium samples were parallel to those produced by pregnenolone. The intrassay and interassay coefficients of variation were 2.3% (n = 6) and 3.7% (n = 4), respectively.

RIA of progesterone. The progesterone concentration in medium samples was measured by RIA as described elsewhere using antiprogesterone serum W-5.[23] The sensitivity of the progesterone RIA was 5 pg per assay tube. The intraassay and interassay coefficients of variation were 3.8% (n = 3) and 6.5% (n = 4), respectively.

RIA of corticosterone. The corticosterone concentration in medium samples was determined by RIA as described elsewhere with anticorticosterone PSW#4-9.[24] The sensitivity of the corticosterone RIA was 5 pg per assay tube. The intraassay and interassay coefficients of variation were 3.3% (n = 5) and 8.2% (n = 4), respectively.

RIA of cAMP. The cAMP concentration in ZG cells extracted by ethanol was measured by a RIA developed in our laboratory as described elsewhere.[24,25] The anti-cAMP CV-27 pool was provided by

the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD). Synthetic tyr-cAMP (Sigma) was used for iodination, and unlabeled cAMP (Sigma) served as a standard preparation. The cAMP RIA sensitivity was 2 fmol per assay tube. The intraassay and interassay coefficients of variation were 3.8% (n = 4) and 6.6% (n = 5), respectively.

Statistical Analysis

All data are expressed as the mean ± SEM. The treatment means were tested for homogeneity using ANOVA, and the difference between specific means was tested for significance using Duncan's multiple-range test.[26] A difference between two means was considered statistically significant at a P level less than .05.

RESULTS

Effect of Orchidectomy and Testosterone Replacement on Plasma Aldosterone Concentration in Male Rats

As compared with the intact state, orchidectomy decreased rat plasma testosterone (36 ± 10 *v* 535 ± 59 pg/mL, $P < .01$) and seminal vesicle weight (188 ± 13 *v* 424 ± 11 mg, $P < .01$). Testosterone replacement restored plasma testosterone levels (598 ± 44 pg/mL) and seminal vesicle weight (460 ± 22 mg) in orchidectomized rats. Plasma aldosterone levels were significantly ($P < .05$) higher in orchidectomized rats injected with oil versus intact rats (Fig 1). TP replacement appeared to restore plasma aldosterone levels in orchidectomized rats to the levels observed in intact rats. There were no differences in plasma levels of Na^+ (138 ± 2 to 143 ± 1 mEq/L), K^+ (3.6 ± 0.1 to 3.9 ± 0.1 mEq/L), and Ca^{2+} (9.2 ± 0.3 to 9.9 ± 0.2 mg/dL) among the three groups.

Effect of Testosterone on Basal or ACTH-Stimulated Aldosterone Secretion and cAMP Accumulation in ZG Cells

Testosterone in the range of 10^{-9} to 10^{-7} mol/L caused a significant ($P < .05$ or $P < .01$) inhibition of aldosterone release by rat ZG cells (Fig 2). ACTH (10^{-9} mol/L) increased ($P < .01$) aldosterone release by ZG cells as compared with basal (unstimulated) release. Treatment with combinations of ACTH and different concentrations of testosterone (10^{-9} to 10^{-7} mol/L) resulted in a significant inhibition of ACTH-stimulated release of aldosterone ($P < .05$ or $P < .01$; Fig 2). In the presence of IBMX, ACTH significantly increased cAMP accumulation in ZG cells (Fig 3). Testosterone alone did not change the cAMP content in ZG cells. Testosterone at doses of 10^{-9} to 10^{-7} mol/L significantly ($P < .05$ or $P < .01$) decreased ACTH-stimulated cAMP accumulation in ZG cells (Fig 3).

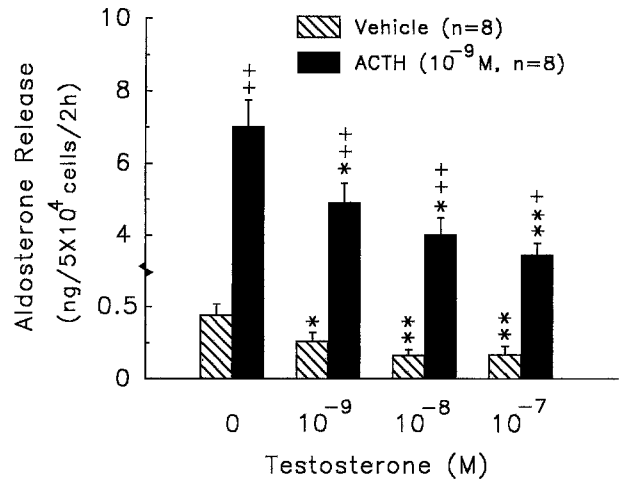

Fig 2. Effect of testosterone (10^{-9} – 10^{-7} mol/L) on basal (unstimulated) and ACTH (10^{-9} mol/L)-stimulated release of aldosterone by ZG cells of male rats. *$P < .05$, **$P < .01$ *v* testosterone 0 mol/L. $^+P < .05$, $^{++}P < .01$ *v* ACTH 0 mol/L (vehicle). Values are the mean ± SEM.

Effect of Testosterone on ANG II-Stimulated Aldosterone Secretion and Steroidogenesis in ZG Cells

Administration of ANG II (10^{-7} mol/L) resulted in a significant increase of aldosterone release ($P < .01$; Fig 4). Testosterone at 10^{-7} mol/L significantly ($P < .01$) suppressed ANG II-stimulated aldosterone release from ZG cells. Corticosterone (10^{-6} or 10^{-5} mol/L; a substrate for aldosterone synthase) markedly increased aldosterone secretion ($P < .05$ or

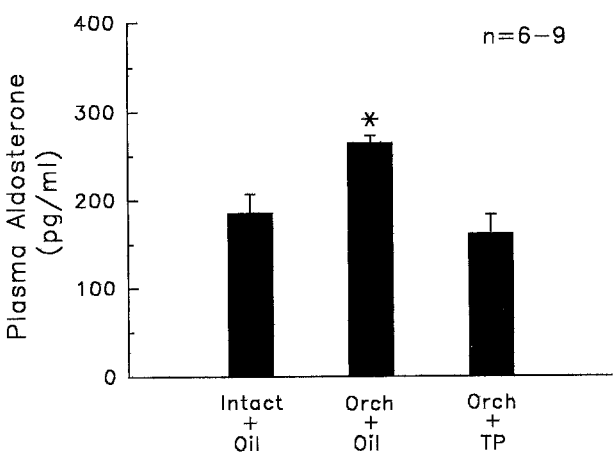

Fig 1. Effect of orchidectomy (Orch) and TP 2 mg/kg body weight (BW) replacement on plasma aldosterone in male rats. *$P < .05$ *v* intact rats. Male rats were orchidectomized for 2 weeks. Orch rats were injected subcutaneously with TP 2 mg/kg BW or sesame oil once daily for 1 week. Values are the mean ± SEM.

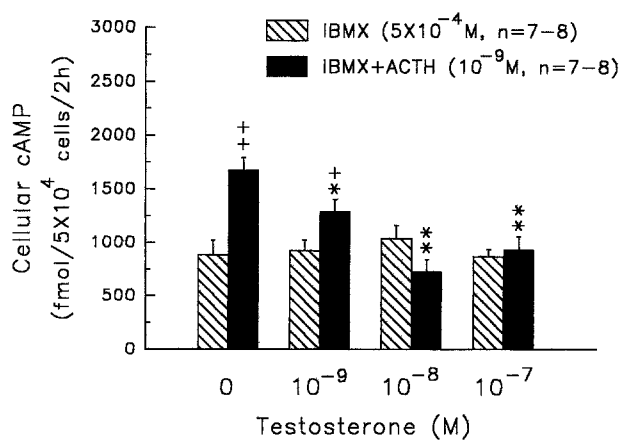

Fig 3. Effect of testosterone (10^{-9} – 10^{-7} mol/L) on basal (unstimulated) and ACTH (10^{-9} mol/L)-stimulated production of cAMP in ZG cells of male rats in the presence of 5 × 10^{-4} mol/L IBMX. *$P < .05$, **$P < .01$ *v* testosterone 0 mol/L. $^+P < .05$, $^{++}P < .01$ *v* ACTH 0 mol/L. Values are the mean ± SEM.

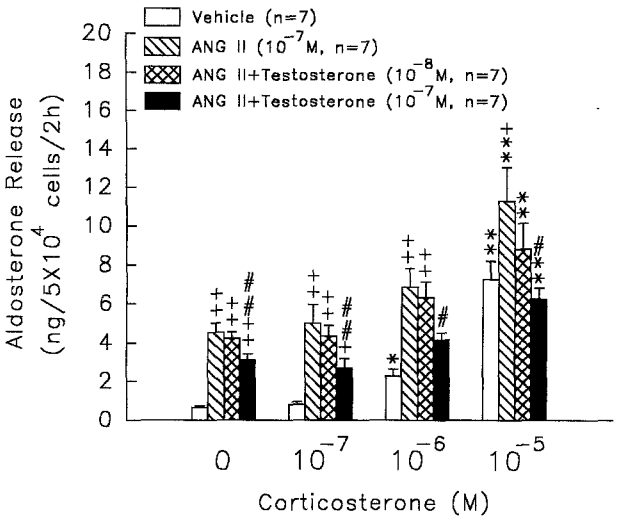

Fig 4. Effect of testosterone (10^{-8} or 10^{-7} mol/L) on ANG II (10^{-7} mol/L)-stimulated aldosterone release by ZG cells of male rats in the presence of corticosterone (0, 10^{-7}, 10^{-6}, or 10^{-5} mol/L). *$P < .05$, **$P < .01$ *v* corticosterone 0 mol/L. $^{+}P < .05$, $^{++}P < .01$ *v* vehicle group. $^{\#}P < .05$, $^{\#\#}P < .01$ *v* ANG II (10^{-7} mol/L) in the absence or presence of corticosterone (10^{-7} – 10^{-5} mol/L). Values are the mean ± SEM.

$P < .01$). In the presence of corticosterone (10^{-5} mol/L), ANG II induced a significantly ($P < .01$) higher increase in aldosterone secretion versus that obtained in the presence of ANG II alone. Testosterone (10^{-7} mol/L) produced an inhibitory effect ($P < .05$ or $P < .01$) on the conversion of corticosterone to aldosterone induced by ANG II plus corticosterone (10^{-7} to 10^{-5} mol/L). Figure 5 shows that administration of corticosterone at 10^{-6} mol/L increased aldosterone release ($P < .01$). Testosterone at 10^{-8} or 10^{-7} mol/L decreased the production of aldosterone from corticosterone in ZG cells.

Incubation of 25-OH-cholesterol, pregnenolone, progesterone, or deoxycorticosterone at 10^{-6} mol/L induced a significant increase (twofold and 16-, 17-, and 34-fold, respectively, $P < .05$ or $P < .01$) in aldosterone secretion by ZG cells as compared with basal release (data not shown). Testosterone at

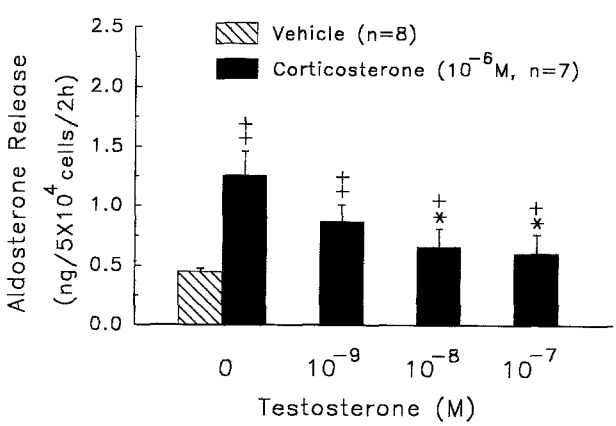

Fig 5. Effect of testosterone (10^{-9} – 10^{-7} mol/L) on corticosterone (10^{-6} mol/L)-enhanced aldosterone release by ZG cells in male rats. *$P < .05$ *v* corticosterone (10^{-6} mol/L). $^{+}P < .05$, $^{++}P < .01$ *v* vehicle. Values are the mean ± SEM.

10^{-7} mol/L significantly decreased all four precursor-stimulated aldosterone release ($P < .05$ or $P < .01$; Fig 6). After incubation with 10^{-6} mol/L 25-OH-cholesterol, pregnenolone, progesterone, or deoxycorticosterone produced an elevation in the production of pregnenolone, progesterone, or corticosterone by ZG cells (data not shown). Testosterone (10^{-8} or 10^{-7} mol/L) resulted in a marked inhibitory effect on the conversion of 25-OH-cholesterol to pregnenolone ($P < .01$; Fig 7). However, there were no effects of testosterone on the conversion of pregnenolone to progesterone, progesterone to corticosterone, or deoxycorticosterone to corticosterone (Fig 7).

DISCUSSION

In the present study, we found that castration increased and testosterone replacement restored the level of plasma aldosterone in male rats. Based on our in vitro data, testosterone appeared to exert a direct inhibitory effect on basal (unstimu-

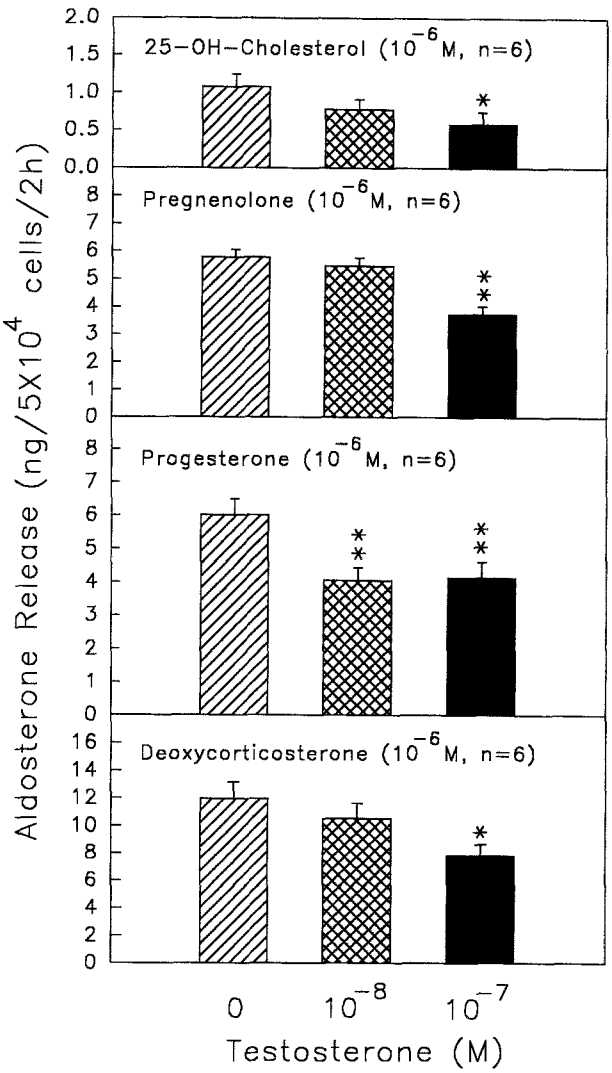

Fig 6. Effect of testosterone (10^{-8} or 10^{-7} mol/L) on 4 different precursor (25-OH-cholesterol, pregnenolone, progesterone, and deoxycorticosterone 10^{-6} mol/L)-stimulated aldosterone release by ZG cells in male rats. *$P < .05$, **$P < .01$ *v* testosterone 0 mol/L. Values are the mean ± SEM.

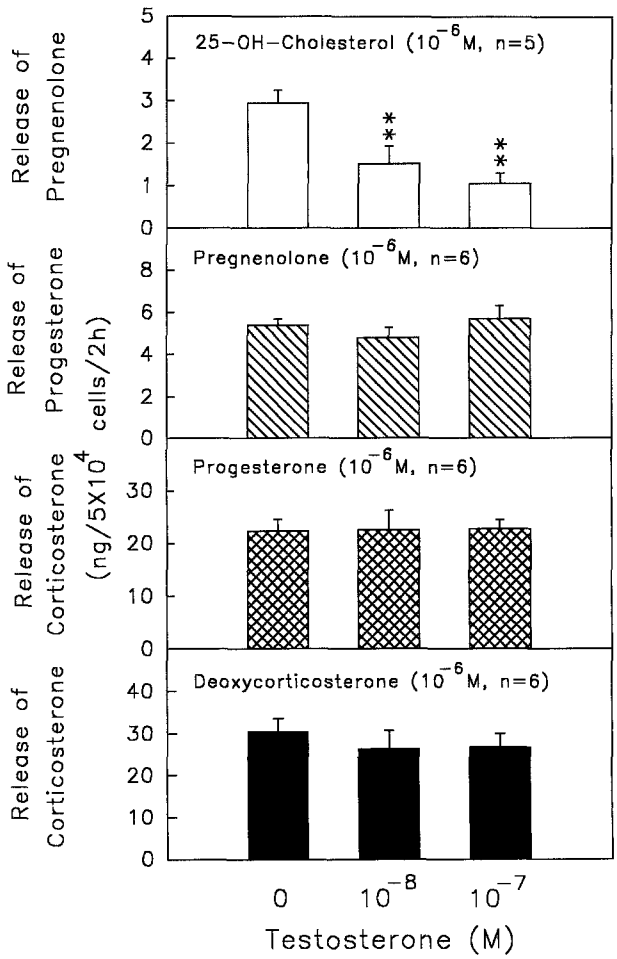

Fig 7. Effect of testosterone (10^{-8} or 10^{-7} mol/L) on the release of pregnenolone, progesterone, and corticosterone in response to 4 different precursors (25-OH-cholesterol, pregnenolone, progesterone, and deoxycorticosterone, 10^{-6} mol/L) in rat ZG cells. **$P < .01$ v testosterone 0 mol/L. Values are the mean ± SEM.

lated) aldosterone release at the level of adrenal ZG cells. It is known that the effects of steroid hormones are mediated by receptors in the nucleus via modulation of transcriptional activity in the responsive cells. However, nongenomic steroid effects in various cells such as neural tissue,[17] Sertoli cells,[18] and vascular smooth muscle cells[19] have also been reported. Gorczynska and Handelsman[18] demonstrated that testosterone rapidly increased cytosolic calcium in Sertoli cells, and suggested that changes in intracellular calcium might be involved in the known synergism between follicle-stimulating hormone and testosterone actions on Sertoli cells. Our present findings show that testosterone had an inhibitory effect on basal aldosterone secretion in rat ZG cells but no effect on the basal level of intracellular cAMP.

Hyperandrogenism often occurs in disorders such as adrenal hyperplasia, adrenal carcinoma, and polycystic ovarian syndrome. Clinical observations in hyperandrogenic women indicated that circulating androgen may inhibit adrenal 21- and/or 11β-hydroxylase activity.[27] It has been shown that androgens in vitro depress the activity of 3β-HSD,[28] 21-hydroxylase,[29] and 11β-hydroxylase,[30] and may be responsible for the decreased production of corticosterone. However, the role of testosterone in aldosterone secretion remains unclear. Several enzymes are involved in the biosynthetic pathway of aldosterone in adrenal ZG cells, eg, P450scc (conversion of cholesterol to pregnenolone), 3β-HSD (conversion of pregnenolone to progesterone), 21-hydroxylase (conversion of progesterone to deoxycorticosterone), 11β-hydroxylase (conversion of deoxycorticosterone to corticosterone), and aldosterone synthase (conversion of corticosterone to aldosterone).[4,31] The present data indicate that the conversion of 25-OH-cholesterol to pregnenolone and of corticosterone to aldosterone was blunted by testosterone. However, testosterone did not alter the conversion of other precursors (pregnenolone, progesterone, or deoxycorticosterone) to progesterone or corticosterone. We therefore suggest that the decrease in aldosterone release by testosterone is partly due to the decreased activity of P450scc and aldosterone synthase.

It is well known that ANG II is an important factor involved in the regulation of aldosterone secretion. ANG II binds to a G-protein–coupled receptor on the surface of cells in the ZG, activating phospholipase C, which hydrolyzes phosphatidylinositol biphosphate, producing inositol triphosphate and diacylglycerol. These substances increase intracellular calcium concentrations and rapidly activate aldosterone biosynthesis via their effects on steroidogenic enzymes.[32] Our in vitro study indicates that testosterone attenuated the aldosterone secretion induced by ANG II and the conversion of corticosterone to aldosterone induced by ANG II in combination with corticosterone in ZG cells. The results of the present study show that testosterone suppressed ANG II-stimulated aldosterone secretion, in part, via inhibition of aldosterone synthase.

In physiological conditions, ACTH is responsible for the increase in plasma aldosterone following acute stress.[4] In the present study, testosterone in vitro had an inhibitory effect on the ACTH-induced increase in aldosterone secretion. This implies that testosterone may have important inhibitory effects on the aldosterone response to stress. Numerous studies have shown that ACTH acts via its G-protein–coupled receptor to activate adenylate cyclase and increase intracellular cAMP levels. Subsequently, cAMP activates the protein kinase A pathway, guiding associated protein complexes to cAMP response elements in the regulatory regions of genes to act as DNA transcription factors. According to our in vitro study, testosterone blunted both aldosterone release and cAMP production in ZG cells in response to ACTH. These results indicate that the inhibitory effect of testosterone on ACTH-stimulated aldosterone release is associated with a decrease of cAMP accumulation. In the presence of IBMX, testosterone at 10^{-8} mol/L completely eliminated ACTH-stimulated cAMP production, whereas testosterone alone at the same dose only partially decreased ACTH-stimulated aldosterone release. The detailed mechanisms are unknown at the present time. Other signaling pathways have been reported in the effect of ACTH on aldosterone secretion, including a sustained increase of calcium influx[33] and an increase of mRNA for steroidogenic acute regulatory (StAR) protein followed by an increase of StAR protein in ZG.[34] Therefore, the finding that testosterone completely inhibited ACTH-stimulated cAMP accumulation but

partially decreased ACTH-stimulated aldosterone secretion may be due to the inefficiency of testosterone in other signaling pathways induced by ACTH. However, more evidence is needed to prove this hypothesis. In contrast to acute stress, chronic stress induces a decrease in aldosterone secretion via inhibition of the late steroidogenic pathway.[35] Long-term administration of ACTH for 9 days induced an increase in StAR mRNA and a decrease in 3β-HSD and P450aldo, as well as unchanged levels of P450scc and P450C21 mRNA.[34] Taken together, these results can explain the different effects on aldosterone secretion induced by different stress states.

In summary, the present study demonstrates that castration induced a higher level of aldosterone, and replacement with testosterone reversed this result in male rats. In the in vitro study, testosterone inhibited the basal and ANG II- and ACTH-stimulated release of aldosterone, via inhibition of P450scc and aldosterone synthase activities, and ACTH-stimulated cAMP accumulation in ZG cells. The inhibitory effect of testosterone on aldosterone release provides information about the correlation between hyperandrogenism and adrenal dysfunction.

ACKNOWLEDGMENT

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REFERENCES

1. Aguilera G, Catt KJ. Regulation of aldosterone secretion during altered sodium intake. J Steroid Biochem 19:525-530, 1983

2. Muller J: Final steps of aldosterone biosynthesis: Molecular solution of a physiological problem. J Steroid Biochem Mol Biol 45:153-159, 1993

3. Quinn SJ, Williams GH: Regulation of aldosterone secretion. Annu Rev Physiol 50:409-426, 1988

4. Aguilera G: Factors controlling steroid biosynthesis in the zona glomerulosa of the adrenal. J Steroid Biochem Mol Biol 45.147-151, 1993

5. Vernikos J, Dallman MF, Keil LC, et al: Gender differences in endocrine responses to posture and 7 days of −6° head-down bed rest. Am J Physiol 264:E153-E161, 1993

6. Critchlow V, Liebelt RA, Bar-Sela M, et al. Sex difference in resting pituitary-adrenal function in the rat. Am J Physiol 205:807-815, 1963

7. Handa RJ, Burgess LH, Kerr JE, et al. Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. Horm Behav 28:464-476, 1994

8. Kitay JI: Pituitary-adrenal function in the rat after gonadectomy and gonadal hormone replacement. Endocrinology 73:253-260, 1963

9. Klett C, Hellmann W, Hackenthal E, et al: Modulation of tissue angiotensinogen gene expression by glucocorticoids, estrogens, and androgens in SHR and WKY rats. Clin Exp Hypertens 15·683-708, 1993

10. Wagner D, Metzger R, Paul M, et al Androgen dependent and tissue specificity of renin messenger RNA expression in mice. J Hypertens 8:45-52, 1990

11. Handa RJ, Nunley KM, Lorens SA, et al: Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. Physiol Behav 55:117-124, 1994

12. Bingaman EW, Magnuson DJ, Gray TS, et al: Androgen inhibits the increases in hypothalamic corticotropin-releasing hormone (CRH) and CRH-immunoreactivity following gonadectomy. Neuroendocrinology 59:228-234, 1994

13. Skelton FR: Production of hypertension, nephrosclerosis and cardiac lesions by methylandrostenediol treatment in the rat. Endocrinology 53:492-505, 1953

14. Salgado E, Selye H: The role of the adrenals in the production of cardiovascular and renal changes by methylandrostenediol. Arch Int Physiol 62:352-358, 1954

15. Gallant S, Alfano J, Charpin M, et al: Expression of adrenal cytochromes P-450 in testosterone-induced hypertension. Hypertension 18:523-528, 1991

16. Rifka SM, Cutler GB Jr, Sauer MA, et al: Rat adrenal androgen receptor: A possible mediator of androgen-induced decrease in rat adrenal weight. Endocrinology 103:1103-1110, 1978

17 Swenson KL, Sladek CD: Gonadal steroid modulation of vasopressin secretion in response to osmotic stimulation. Endocrinology 138:2089-2097, 1997

18. Gorczynska E, Handelsman DJ: Androgens rapidly increase the cytosolic calcium concentration in Sertoli cells. Endocrinology 136:2052-2059, 1995

19. Farhat MY, Abi-Younes S, Dingaan B, et al: Estradiol increases cyclic adenosine monophosphate in rat pulmonary vascular smooth muscle cells by a nongenomic mechanism J Pharmacol Exp Ther 276:652-657, 1996

20. Purdy SJ, Whitehouse BJ, Abayasekara DRE· Stimulation of steroidogenesis by forskolin in rat adrenal zona glomerulosa preparations. J Endocrinol 129:391-397, 1991

21. Kau MM, Lo MJ, Tsai SC, et al: Effects of prolactin on aldosterone secretion in rat zona glomerulosa cells. J Cell Biochem 72:286-293, 1999

22. Wang PS, Tsai SC, Hwang GS, et al: Calcitonin inhibits testosterone and luteinizing hormone secretion through a mechanism involving an increase in cAMP production in rats. J Bone Miner Res 9:1583-1590, 1994

23. Lu SS, Lau CP, Tung YF, et al: Lactate stimulates progesterone secretion via an increase in cAMP production in exercised female rats. Am J Physiol 271:E910-E915, 1996

24. Lo MJ, Kau MM, Chen YH, et al: Acute effects of thyroid hormones on the production of adrenal cAMP and corticosterone in male rats. Am J Physiol 274:E238-E245, 1998

25. Tsai SC, Lu CC, Lau CP, et al: Progesterone stimulates in vitro release of prolactin and thyrotropin involving cAMP production in rat pituitary. Chin J Physiol 39:245-251, 1996

26. Steel RGD, Torrie JH: Principles and Procedures of Statistics New York, NY, McGraw-Hill, 1960

27. Givens JR, Andersen RN, Ragland JB, et al: Adrenal function in hirsutism. I. Diurnal change and response of plasma androstenedione, testosterone, 17-hydroxyprogesterone, cortisol, LH and FSH to dexamethasone and ½ unit of ACTH. J Clin Endocrinol Metab 40:988-1000, 1975

28. Yates J, Deshpande N: Kinetic studies on the enzymes catalysing the conversion of 17α-hydroxy-progesterone and dehydroepiandrosterone to androstenedione in the human adrenal gland in vitro J Endocrinol 60:27-35, 1974

29. Hornsby PJ: Regulation of 21-hydroxylase activity by steroids in cultured bovine adrenocortical cells: Possible significance for adrenocortical androgen synthesis. Endocrinology 111:1092-1101, 1982

30. Baird A, Kan KW, Solomon S: Androstenedione-mediated

inhibition of 11β-hydroxylation in monolayer cell cultures of fetal calf adrenals. J Steroid Biochem 18:581-584, 1983

31. Simpson ER, Waterman MR: Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. Annu Rev Physiol 50:427-440, 1988

32. Curnow KM, Tusie-Luna MT, Pascoe L, et al: The product of the CYP11B2 gene is required for aldosterone biosynthesis in the human adrenal cortex. Mol Endocrinol 5:1513-1522, 1991

33. Kojima I, Kojima K, Rasmussen H: Role of calcium and cAMP in the action of adrenocorticotropin on aldosterone secretion. J Biol Chem 260:4248-4256, 1985

34. Lehoux JG, Fleury A, Ducharme L: The acute and chronic effects of adrenocorticotropin on the levels of messenger ribonucleic acid and protein of steroidogenic enzymes in rat adrenal in vivo. Endocrinology 139:3913-3922, 1998

35. Aguilera G, Kiss A, Sunar-Akbasak B: Hyperreninemic hypoaldosteronism after chronic stress in the rat. J Clin Invest 96:1512-1519, 1995