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Testosterone Effect on Renin System in Rats[1] (39800)

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The effect of estrogens on the renin-angiotensin system (RAS) have been well delineated. Thus, in both humans (1) and rats (2) estrogens stimulate the synthesis of angiotensinogen, the renin substrate (RS). Consequently there ensues an increase in plasma renin activity (PRA) in both species (1, 3). The resulting increase in plasma angiotensin II (4) has a negative feedback effect on the renal secretion of renin (5), and plasma renin concentration (PRC) falls significantly in both species (3, 4). This results in a final PRA during estrogen treatment which is not significantly different from control levels in the rat (3). In the human, however, the negative feedback system is apparently not as effective since PRA during estrogen therapy remains somewhat elevated (1, 4, 6).

Androgens have the opposite effects from estrogens on the level of the special-function proteins made in the liver, such as thyroxine binding globulin (7) and corticosteroid binding globulin (8). The effect of androgens on RS and the rest of the RAS, however, has not been extensively studied. Nasjletti *et al.* (9) reported that castration had no effect on RS levels in male rats and that 10 mg of testosterone given for 21 days had no effect on RS in ovariectomized rats. A preliminary report showed a temporary reduction in PRA with testosterone treatment in men (10).

The purpose of the present study was to delineate the alterations, if any, in the RAS following the administration of testosterone to rats.

Materials and methods. Animal studies. Sprague-Dawley rats from the Sprague-Dawley Co., Madison, Wisconsin, were used throughout. In experiment 1 animals were ovariectomized under ether anesthesia at age 15 weeks and treatment was begun 1 week later. For experiment 2, 50-day-old rats were orchidectomized and treatment was begun 1 week later. In experiment 3 weanlings were orchidectomized at 26 days. Microscopic examination of the testes revealed absence of spermatogenesis and of apparent Leydig cells. Treatment of these rats was begun 1 month after orchidectomy.

All experimental rats were given 2.5 mg of testosterone (Upjohn; 25 mg/ml) im daily for 21 days. In experiment 1 controls were untreated. In experiments 2 and 3 controls received a vehicle of carboxymethylcellulose, polysorbate 80, sodium chloride, benzyl alcohol, and water. The rats were sacrificed on the day after injections ended by guillotine decapitation without prior agitation of the animals. Preliminary experiments confirmed that anesthesia, stunning, or heart puncture increased PRA (11, 12). The rats' blood was collected on ice in tubes containing disodium EDTA, 1 mg/ml, as anticoagulant. All plasma was frozen until time of assay. Body weights of all rats were recorded and so were the weights of the seminal vesicles in the males.

Renin-angiotensin system. Preparation of rat renin. Renin was extracted from 120 g of frozen rat kidneys by the method of Sen *et al.* (13). The unit of rat renin for the purpose of this study was defined in reference to hog renin obtained from Nutritional Biochemicals ICN, Cleveland, Ohio. The hog renin was calibrated in Goldblatt units in which 1 U has a 30- to 35-mm pressor effect in a dog (14). Since 1 Goldblatt μU of the hog renin generated 0.06 ng of angiotensin I (AI) when incubated with 0.04 ml of normal rat plasma for 3 hr, the unitage of our rat renin was defined in the same way. Although one might expect a unit of our rat renin to be more highly pressor in the rat than a unit of hog renin, it required 0.156 U

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of rat renin for 7–7.5 mm Hg blood pressure increase in the rat compared to 0.05 U of hog renin (kindly performed by Dr. Keith McDonald). The yield of rat renin from kidney tissue was 1.7 U/g net weight, similar to the value of 2.2 reported by Haas *et al.* (15).

PRC and PRA. For PRC determination a method analogous to the enzyme kinetic technique previously described in our laboratory for humans (16) was utilized. The pH of 2 ml of plasma was adjusted to 6.5, the optimum pH for rat renin acting on rat RS, with 1 *N* HCl, using an automatic titrator (Radiometer). As an angiotensinase inhibitor, 1/20 vol of phenylmethylsulfonyl fluoride, 50 mg/ml of ethanol, was added. Aliquots of 0.2 ml of plasma were incubated for 1 hr at 37° with 0.05 ml of 0.2 *M* ammonium acetate buffer, pH 6.5, containing 0, 16, 32, and 48 μU of added rat renin. One aliquot without added renin was incubated at 0°. Duplicate aliquots of 0.05 ml from each incubation tube were assayed for AI by the direct radioimmunoassay method previously described (17), except that γ counting was performed on the charcoal residues using a γ scintillation spectrometer. The value of PRA was computed from the tubes containing no added renin. Utilizing a specially written Algol program for a Burroughs B6700 time-sharing computer, a straight-line least-squares plot was obtained for the net angiotensin generated in each aliquot assayed plotted against the amount of exogenous renin added. PRC was then computed as PRA divided by the slope of that line. A typical plot of a PRC assay in which the usual as well as intermediate doses of added renin were used is shown in Fig. 1.

Renin substrate. RS was measured in rat plasma by incubating 0.2 ml of plasma, diluted 1:200 with 0.2 *M* ammonium acetate buffer, pH 6.5, with 0.05 ml of the same buffer containing 16500 μU of rat renin for 6 hr at 37°. Preliminary experiments revealed that 4 hr of incubation and 3900 μU of renin were adequate for maximum generation of AI, which was measured by radioimmunoassay against a standard curve in similarly diluted deangiotensinized plasma (17).

Plasma aldosterone. This was measured in duplicate in pooled plasma from 8 to 12

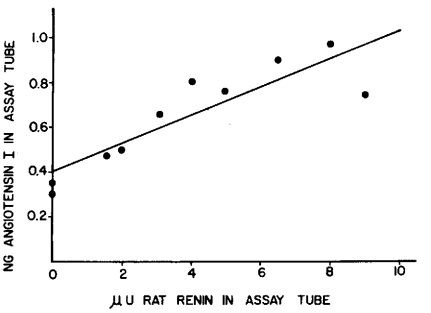

FIG. 1. Plot of actual AI assayed after 37° incubation in tubes containing 0.036-ml aliquots of a rat's plasma and various additions of rat renin. "Endogenous AI," the mean value for two tubes incubated at 0°, which was 0.14 ng, has not been subtracted from any of the values shown. PRA for this sample was 5.0 ng/ml/hr and PRC (PRA/slope) was 81.5 μU/ml.

control or experimental rats in each experiment. The aldosterone was estimated in pools because as little as 0.5 ml of plasma remained of some rats' plasma following the renin determinations. Following extraction from plasma and immunologic purification, aldosterone was measured by radioimmunoassay, as previously described (18). This method recovers 40 to 60% of the aldosterone in the sample, and each sample result is corrected according to recovery of added aldosterone tracer. Preliminary experiments revealed that corticosterone was excluded completely enough in the purification so as not to interfere in the assay.

Results. Testosterone treatment had no significant effect on the body weights of the rats in any of the three experiments. All groups continued to gain weight at the same rate as controls. The body weights at the end of the experiment ranged from 248 to 313 g. In the male rats in both experiments 2 and 3 the weights of the seminal vesicles plus coagulating gland in the treated animals were normal (820–1500 mg), whereas those in the controls were infantile (<90 mg).

The results of the measurements of the elements of the RAS in all three experiments are shown in Table I. In the two experiments on male rats PRA was significantly increased following testosterone treatment ($P < 0.01$). This was not due to estrogen-like action on RS except in experiment 3, in which a significant increase in substrate occurred ($P < 0.05$). In the other

TABLE I. EFFECTS OF TESTOSTERONE ON THE RAS IN CASTRATED RATS.[a]

Castration (age) Age at sacrifice (days)	Experiment 1[b] Ovariectomy, 105 days 133		Experiment 2 Orchidectomy, 50 days 78		Experiment 3 Orchidectomy, 26 days 75	
	Control	Treatment	Control	Treatment	Control	Treatment
PRA (ng of AI/ml/hr)	15.7 ± 1.9 (10)	16.4 ± 9.2 (12)	13.0 ± 6.7 (14)	23.8 ± 10.6 (13)*	6.8 ± 2.7 (10)	12.2 ± 5.4 (13)*
PRC (μU/ml)	456 ± 146 (10)	523 ± 170 (11)	442 ± 322 (14)	672 ± 199 (10)**	226 ± 171 (11)	269 ± 129 (13)
RS (ng of AI/ml)	835 ± 109 (12)	785 ± 69 (12)	792 ± 88 (14)	774 ± 98 (14)	964 ± 146 (13)	1092 ± 122 (12)**

[a] Testosterone, 2.5 mg/day, was given for 21 days to treatment groups.
[b] Mean ± SD (*N*).
* $P < 0.01$ compared to control value.
** $P < 0.05$ compared to control value.

two experiments increased renin secretion, as shown by an increased PRC, appeared to be responsible for the stimulation of PRA. However, only in experiment 2 did this increase in PRC, which was noted in all three experiments, reach statistical significance ($P < 0.05$).

Plasma aldosterone levels, measured in plasma pools obtained from the various groups of rats, were not significantly different between the control and treated animals (Table II).

TABLE II. EFFECT OF TESTOSTERONE ON PLASMA ALDOSTERONE IN POOLS OBTAINED FROM RATS.

Experiment	Group	Plasma aldosterone (pg/ml)
1	Control	187
	Testosterone	196
2	Control	124
	Testosterone	117
3	Control	173
	Testosterone	140
Mean ± SD	Control	163 ± 33
	Testosterone	151 ± 41
P		NS

Discussion. These studies have shown a surprising significant stimulatory effect of testosterone on PRA in castrated young male rats. This appeared to be an effect on renin secretion since PRC, the plasma concentration of the enzyme, was increased by the androgen treatment, albeit only significantly in one of our three experiments. Moreover, in the group of male rats castrated before the onset of gonadal maturation, testosterone therapy was associated with an increased level of RS. This could conceivably be related to conversion of a portion of this large dose (about 10 mg/kg) of testosterone to estrogen and resulting enhancement of RS synthesis by the estrogen.

What other factors might be playing a role in this testosterone-induced increase in renin secretion? Testosterone potentiates renal growth as well as the renal levels of some enzymes (19). Although renal renin has not been shown previously to be under the control of androgens, testosterone (20) has been shown to be an important factor in the level of the renin-like enzyme of the mouse submaxillary gland (21). The analogy of mouse submaxillary gland renin-like enzyme even with its rodent cousin, rat submaxillary gland renin-like activity, is in some doubt, however. For example, release of the renin-like activity in the mouse submaxillary gland is quite responsive to α-adrenergic agonists, whereas the murine renal enzyme responds to the β-adrenergic stimulant isoproterenol which does not affect the submaxillary enzyme (22). Rat submaxillary gland activity, on the other hand, is stimulated by isoproterenol (23), as is true of rat renal renin (24). Chlorothiazide and ouabain also increase rat submaxillary gland renin (23).

Since recent studies have suggested that aldosterone secretion in the rat is more closely responsive to the RAS than previously thought (25), it is possible that androgen effects on sodium balance might be responsible for the testosterone stimulation of PRA. Both competitive (26) and noncompetitive (26, 27) antagonism to mineralocorticoids have been demonstrated for testosterone (26) and adrenocortical androgens (27). In men also, testosterone has been shown to be natriuretic (28). However, the lack of significant change in plasma aldosterone with testosterone treatment in this study would speak against this mode of action of testosterone on renin secretion.

Finally, the present study confirms the

lack of effect of testosterone on RS reported by Nasjletti *et al.* (9) in ovariectomized female rats. The failure to find reductions in PRA in the present study, as previously reported in humans (10), could well be due to species differences or the different time of measurement.

Summary. Testosterone, 2.5 mg/day, was administered for 21 days to groups of female rats ovariectomized at age 105 days, male rats orchidectomized at age 50 days, and male rats orchidectomized as 26-day-old weanlings. The three groups were sacrificed at 133, 78, and 75 days, respectively, at body weights ranging from 248 to 313 g. In the two male groups there was a significantly greater plasma renin activity in testosterone-treated animals ($P < 0.01$) than in untreated controls. Plasma renin concentration rose with treatment in all three groups but only in the older males was this increase statistically significant ($P < 0.05$). RS was unchanged with treatment, however, except in the youngest group of males ($P < 0.05$). This could have been due to conversion of testosterone to estrogens. Plasma aldosterone was not significantly altered by testosterone treatment. These results suggest that testosterone stimulates secretion of renin in at least the young male rat. Since testosterone has previously been shown to stimulate levels of the renin-like enzyme in mouse submaxillary gland, it is unclear from these experiments whether renal or submaxillary renin is involved. Although an indirect mechanism of antagonism by testosterone to action of mineralocorticoids could be playing a role, this is unlikely because aldosterone was unchanged.

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