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Effect of Testosterone on Carbonic Anhydrase and MG^{2+}-Dependent HCO_3^--Stimulated ATPase Activities in Rat Kidney: Comparison with Estradiol Effect

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ABSTRACT. Effects of testosterone administration (TP; 1–3 mg/kg body weight, SC, once daily for 7 days) on the cytosol carbonic anhydrase (CA) and tubular brush border Mg^{2+}-dependent HCO_3^--stimulated ATPase (Mg^{2+}–HCO_3^-–ATPase) activities of normal and castrated male and female rat kidney were compared with estradiol (E_2) effects. TP decreased kidney CA activity in a dose-dependent manner in all four animal conditions, and negative correlations were observed between cytosol CA activity and serum testosterone concentration. However, brush border Mg^{2+}–HCO_3^-–ATPase activity was not affected by testosterone. Orchiectomy increased only CA activity and its value recovered to normal levels by 3 mg/kg TP replacement, whereas Mg^{2+}–HCO_3^-–ATPase activity did not change. In the previous study, on the other hand, E_2 administration activated both kidney enzymes in normal male rats and only Mg^{2+}–HCO_3^-–ATPase in normal female rats. Testosterone and E_2 conversely affected kidney CA activity in male rats. These facts suggest that the cytosol CA may control kidney functions on H^+ and HCO_3^- metabolism under the balance of both sex hormones in the living body. COMP BIOCHEM PHYSIOL 114C;2:105–112, 1996.

KEY WORDS. Brush border, carbonic anhydrase, cytosol, estradiol, hormonal regulation, Mg^{2+}–HCO_3^-–ATPase, rat kidney, testosterone

INTRODUCTION

Carbonic anhydrase (CA) was first isolated from red blood cells (40). It catalyzes the reversible hydration of carbon dioxide and also functions in the transfer of H^+ and HCO_3^- in secretory epithelium (39,46). Recently, the presence of several CA isozymes, CA-I–CA-VII, identified by the immunohistochemical method or purification procedures, has been reported in many organs (11,13,18,58). Further, the genetic expression of each isozyme has been reported (59).

In the kidney, presence of three CA isozymes, II, IV, and V, and their physiological functions have been reviewed (14,49). CA-II exists in the cytosol of renal tubular cells and hydrates the CO_2 to H_2CO_3, which in turn dissociates to HCO_3^- and H^+. The HCO_3^- generated in the cytosol is thought to be transported from the cytosol to the interstitial fluid, and the H^+ can be secreted into lumen in exchange for Na^+ (13,48). CA-IV is highly expressed on the apical brush border membrane (and somewhat on the basolateral membrane) of proximal tubular cells and thought to be primarily responsible for urinary bicarbonate resorption through H^+–HCO_3^- transport (6,17,62). CA-V is present in mitochondria and has a role for ureagenesis and gluconeogenesis (15,16).

Another characteristic of CA is that the enzyme activities or its concentrations in tissues are influenced by several hormones. These phenomena have been demonstrated first by Lutwak-Mann (37), and thereafter many investigators have challenged this theme. Recently, effects of sex hormones on CA-II and CA-III concentrations in the liver (7,24,47) and the prostate (20,21) have been reported, indicating that the inducing or decreasing effects of androgens on tissue CA concentrations are different relating to its isozyme types; however, regulation of kidney cytosol CA-II activity by testosterone has not been demonstrated.

The presence of Mg^{2+}–HCO_3^-–ATPase activity in the microsomal and mitochondrial fractions of rat small intestinal mucosa and proximal tubular cells was reported 30 years ago. This ATPase in the brush border membrane of rat kidney tubular cells was further reported (30,31) and thought to be related to membrane transport of H^+ and HCO_3^- through exchange process. Nevertheless, there are still many opinions related to its localization and physiological functions (1,19,33,50).

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CA-II and CA-IV in renal tubular cells have an important role for generation of HCO_3^-. This anion also stimulates H-ATPase activity in apical membrane resulting in the acceleration of H^+ transport. Therefore, it is hypothesized that the activation of CA may stimulate the HCO_3^-–ATPase activity and also the reverse may occur; however, the coupling of this ATPase and CA on membrane transport of H^+ and HCO_3^- in the kidney tubule has not been elucidated.

Testosterone is reduced to 5 α-dihydrotestosterone (an active form of testosterone) by 5 α-reductase in cytoplasm of prostate and seminal vesicle of rodents, binds with its receptors and appears the acceleration of the physiological functions (23,61). Unlike these androgen-responsive cells, renal cells have low 5 α-reductase activity, and testosterone directly binds to androgen receptors and induces several enzymes (4,8,34). Ornithine decarboxylase (45) and β-glucuronidase (3,4,57) are well known as striking markers of androgen action among several enzymes in mouse kidney. On the other hand, effects of testosterone on CA and Mg^{2+}–HCO_3^-–ATPase activities in rat kidney have not been investigated.

We have recently shown that the specific activities of cytosol CA and brush border Mg^{2+}–HCO_3^-–ATPase in male rat kidney cortex were not affected by adrenalectomy and adrenocorticoids administration (52) and thyroidectomy and T_4 administration (53). On the other hand, administration of estradiol-17β (E_2) to male rats exhibited the activities of both CA and Mg^{2+}–HCO_3^-–ATPase in the kidney cortex, although brush border Mg^{2+}–HCO_3^-–ATPase activity in female rat kidney cortex was only increased by E_2 administration with no effect on CA activities, indicating a sex difference between males and females (55). Therefore, to examine the effect of testosterone on above enzyme activities in male and female rat kidney, it may be interesting to clarify the molecular mechanism of action of sex hormones on kidney functions.

MATERIALS AND METHODS

Animals

Male and female Wistar rats (*Rattus norvegicus albus* 7–8 weeks old and weighing 200–250 g in males and 150–180 g in females) were purchased from the Shizuoka Agricultural Cooperative Association for laboratory animals (Hamamatsu, Shizuoka, Japan). Before the experiments, all animals were housed at room temperature (approximately 24°C) with a 12-hr light-darkness cycle and were maintained on a commercial solid diet with tap water *ad libitum* for 1 week in the University Animal Center. Orchiectomy and oophorectomy were performed under pentobarbital anaesthesia (Nembutal sodium, Abbott Laboratories, North Chicago, IL, U.S.A.; 20 mg/kg body weight [BW], IP).

Chemicals

An oily injection of testosterone propionate (TP; Enarmon, Teikokuzoki Pharmaceutical Co., Tokyo, Japan) was diluted with sesame oil to appropriate concentrations and injected subcutaneously (SC), once daily for 1 week in 0.1 ml solvent/100 g BW. Controls or sham-operated controls were treated with the same volume of sesame oil. Disodium adenosine-5'-triphosphate (Na_2ATP; Boeringer, Mannheim, Germany) was dissolved in distilled water and brought to pH 8 with Tris (hydroxymethyl)-aminomethane. Other chemicals used were reagent grade.

Experimental Procedure

All animals were killed between 10:00 and 15:00 on the day of experiment. At 24 hr after the last injection of TP, rats were anesthetized with pentobarbital (30 mg/kg, IP), and blood (about 4 ml) was taken from the femoral artery into glass test tubes and left for 4–5 hr in a refrigerator. After coagulation of the blood, serum was separated by centrifugation at 1870 **g** for 10 min and analyzed by hormone assay. Immediately after the collection of blood sample, the abdomen was opened and the whole body was perfused with cold heparinized saline through the portal vein and the abdominal aorta. Thereafter, the kidneys were removed, decapsulated and pieces of the cortex were washed with cold saline, pooled and weighed. Prostate and uterus were also taken out and weighed.

The crude samples of CA- and Mg^{2+}–HCO_3^-–ATPase-containing fractions were prepared by a calcium precipitation procedure similar to those described by Malathi *et al.* (38). In brief, each kidney cortex was homogenized in a Potter-Elvehjem type glass Teflon homogenizer with 10 ml of ice-cold 50 mM D-mannitol, 2 mM Tris (hydroxymethyl)-aminomethane–HCl buffer (pH 7.1 at 4°C). Homogenates were filtered through a piece of nylon mesh, and aliquots of this homogenate were taken for the determination of enzyme activity and protein content. Thereafter, $CaCl_2$ was added to the residual homogenate to a final concentration of 10 mM and the mixture was gently stirred in an ice water bath for 10 min. The homogenates were then centrifuged at 3000 **g** for 15 min in a refrigerated centrifuge. The supernatant was carefully decanted and centrifuged at 43,000 **g** for 20 min. The resulting supernatant was decanted and used as a CA-containing sample. Pellets were rehomogenized in the same mannitol–Tris buffer and recentrifuged at 43,000 **g** for 20 min. The supernatant was decanted and pellets (crude brush border-containing fraction) were homogenized with the same buffer and used as the Mg^{2+}–HCO_3^-–ATPase-containing sample. All samples were stored at −20°C until use.

Recently, we purified rat kidney cytosol CA obtained from 43,000 **g** centrifugation by using Sephadex G-75 gel filtration and DEAE Sephadex A-50 ion exchange column chromatography and confirmed that the purified CA-containing fraction showed only a single band having a molecular weight of 30 kDa by SDS-polyacrylamide slab gel electrophoresis (Suzuki, unpublished data). The electrophoretic mobility is consistent with CA-II.

CONTAMINATION OF BLOOD TO KIDNEY CYTOSOL. To account for the contamination of erythrocytes destroyed by kidney homogenization, the hemoglobin released into the kidney cytosol fraction was spectrophotometrically examined at 577 nm (22) as oxyhemoglobin (HbO_2). Blood CA (CA-I + CA-II) activity contaminated into cytosol CA was calculated based on the HbO_2 concentration. Contamination of blood CA was about 10% of cytosol CA.

CONTAMINATION OF MITOCHONDRIA TO BRUSH BORDER MEMBRANE. We reported previously (54) that the isolated brush border from rat kidney by Ca precipitation procedure (38) contained no succinate dehydrogenase activity. Therefore, brush border membrane in the present experiment also may not be contaminated with mitochondria.

Assay of Enzyme Activity

CARBONIC ANHYDRASE. Carbonic anhydrase activities in the homogenate and cytosol fractions were assayed by the colorimetric method of Wilbur and Anderson (60). Enzyme activity was calculated according to the following expression (17): enzyme activity unit = $(T_0 - T)/T$ per mg protein, where T_0 is the reaction time for the uncatalyzed reaction and T represents the time interval for the catalyzed reaction.

Mg^{2+}–HCO_3^-–ATPase. Mg^{2+}-dependent, HCO_3^--stimulated ATPase activity was assayed as described previously (51). Aliquots of samples were placed in test tubes (15 × 100 mm) with final concentrations of 1.0 mM $MgCl_2$, 50 mM $NaHCO_3$, 3 mM ATP and 50 mM Tris–HCl buffer (pH 9.0 at 37°C) in a total volume of 1.0 ml. After incubation at 37°C for 10 min in a shaking water bath, the test tubes were chilled in ice water and the reaction was stopped by the addition of 1 ml 20% (w/v) trichloroacetic acid. The precipitates were removed by centrifugation at 1870 **g** for 10 min, and the released inorganic phosphate (Pi) in the supernatant was determined by the method of Allen (2) as modified by Nakamura (44). Enzyme activity was expressed as μM Pi/mg protein/hr. Mg^{2+}-ATPase activity was assayed in the absence of additional $NaHCO_3$.

Protein Assay

Protein in the enzyme samples was determined by the method of Lowry *et al.* (36) with bovine serum albumin as a standard.

Hormone Assay

Serum hormone concentrations were assayed by radioimmunoassay (RIA) using the following commercial assay kits. Testosterone (T) RIA kit (Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) using a double-antibody method, which has a minimal detection limit of 40 pg/ml and intra- and interassay coefficients of variation of between 4.4 and 8.7% and between 9.2 and 12.9%, respectively. E_2 direct RIA kit (Sorin Biomedica STA, Saluggia, VC, Italy) using a double-antibody method that has a minimal detection limit of 4.5 pg/ml and intra- and interassay coefficients of variation of between 2.7 and 4.9% and between 4.9 and 5.7%, respectively. Aldosterone RIA kit (Dainabot Company, Tokyo, Japan) using a double-antibody method that has a minimal detection limit of 13.5 pg/ml and intra- and interassay coefficients of variation of between 4.5 and 9.9% and between 4.5 and 13.3%, respectively. Cortisol RIA kit (Dainabot Company, Tokyo, Japan) using a double-antibody method that has a minimal detection limit of 20 ng/ml and intra- and interassay coefficients of variation of between 2.7 and 4.5% and between 2.4 and 4.9%, respectively. The cross-reactivity of antibody of this cortisol RIA kit with cortisol and corticosterone was 100 and 44.1%, respectively. As corticosterone is the major glucocorticoid in rats, a greater part of cortisol measured by this kit is actually corticosterone; therefore, we expressed corticosterone concentration as cortisol-like material concentration.

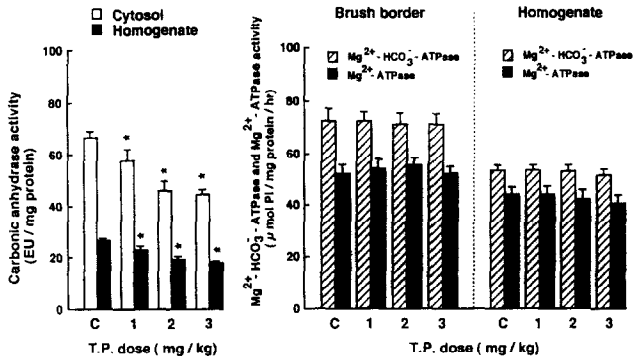

FIG. 1. Effects of TP administration on CA activities in cytosol and homogenate (lefthand side) and Mg^{2+}–HCO_3^-–ATPase and Mg^{2+}–ATPase activities in brush border and homogenate (righthand side) of normal male rat kidney. Each column represents the mean ± SD. $n = 5$. *Statistically significant compared with control (C) ($P < 0.01$).

Statistical Analysis

All values presented are means ± SD. Differences between means for corresponding data were evaluated statistically by using the one-way analysis of variance followed by the Scheffe's multiple comparison test. All calculations including linear regression analysis were carried out using the computer program (StatView IV, Abacus Concept. Inc., Berkeley, CA, U.S.A.). A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Effects of the Administration of TP to Normal Male Rats

Figure 1 shows the effects of TP administration in doses of 1–3 mg/kg BW/d, SC, for 7 days on the kidney enzyme

TABLE 1. Effects of TP administration on organ weight and serum hormone concentration under various animal conditions

Group	Organ weight (mg/100g BW)				Serum hormone concentration			
	Adrenal	Kidney	Prostate	Uterus	T (nM/l)	E_2 (nM/l)	Aldosterone (nM/l)	Cortisol (μM/l)
Male control (n = 5)	16.5 ± 1.6	667 ± 70	246 ± 24		9.98 ± 2.58			
+TP 1 mg/kg (n = 5)	16.7 ± 1.7	655 ± 40	303 ± 8**		12.08 ± 3.14			
+TP 2 mg/kg (n = 5)	18.0 ± 2.0	684 ± 56	314 ± 27**		16.47 ± 3.54**			
+TP 3 mg/kg (n = 5)	16.8 ± 1.6	666 ± 27	327 ± 7**		26.71 ± 4.52**			
Sham-op male control (n = 8)	14.1 ± 1.6	649 ± 26	235 ± 39		13.44 ± 4.92		0.40 ± 0.10	0.35 ± 0.07
Orchiectomy 2 weeks (W) (n = 8)	20.5 ± 1.9**	637 ± 58	30 ± 8**		0.53 ± 0.12**		0.18 ± 0.03**	0.19 ± 0.10**
Orchi. 1 W + TP 3 mg/kg (n = 8)	18.3 ± 1.1†	693 ± 72	147 ± 23††		26.17 ± 4.48††		0.31 ± 0.08††	0.35 ± 0.08††
Female control (n = 7)	31.2 ± 5.1	726 ± 90		221 ± 52	<0.14	0.11 ± 0.06		
+TP 1 mg/kg (n = 7)	31.9 ± 5.0	732 ± 64		213 ± 34	5.97 ± 1.22	0.10 ± 0.06		
+TP 2 mg/kg (n = 7)	30.3 ± 3.8	723 ± 42		274 ± 38	13.35 ± 3.79**	0.09 ± 0.05		
+TP 3 mg/kg (n = 7)	30.3 ± 3.1	731 ± 38		293 ± 39*	23.00 ± 7.31**	0.11 ± 0.04		
Sham-op female control (n = 6)		698 ± 33		240 ± 40	<0.14	0.21 ± 0.11 (3)		
Oophorectomy 2 weeks (W) (n = 6)		670 ± 21		52 ± 8**	<0.14	0.05 ± 0.01 (3)**		
Oophor. 1 W + TP 1 mg/kg (n = 6)		710 ± 30		100 ± 14††	5.23 ± 0.67††	0.05 ± 0.03 (3)		
Oophor. 1 W + TP 2 mg/kg (n = 6)		720 ± 39		126 ± 17††	11.98 ± 3.00††	0.09 ± 0.07 (3)		
Oophor. 1 W + TP 3 mg/kg (n = 6)		761 ± 12		156 ± 8††	16.61 ± 4.05††	0.05 ± 0.01 (3)		

Values are means ± SD.
*, **Statistically significant compared with controls (*$P < 0.05$, **$P < 0.01$).
†, ††Statistically significant compared with castrated rats (†$P < 0.05$, ††$P < 0.01$).

activities. CA activities (lefthand side) in cytosol and homogenate were decreased after TP administration in a dose-dependent manner. The following negative correlation was observed between cytosol CA activity (y) and serum T concentration (nM/1, x):

$$y = -0.977x + 69.535, \quad r = -0.739, n = 20,$$

$$P < 0.01.$$

On the other hand, Mg^{2+}–HCO_3^-–ATPase and Mg^{2+}-ATPase activities in brush border and homogenate (righthand side) did not change after TP administration.

Table 1 shows the effects of TP administration on adrenal, kidney, total prostate and uterus weights and serum hormone concentrations in various animal conditions. After TP administration to normal male rats, serum T concentration and prostate weight were increased in a dose-dependent manner. Body weight was not changed pre- and post-TP administration (data not shown).

Effects of the Administration of TP to Orchiectomized Rats

Figure 2 shows the effects of orchiectomy and TP replacement (3 mg/kg BW/day SC, for 7 days from 1 week after operation). CA activities (lefthand side) in cytosol and ho-

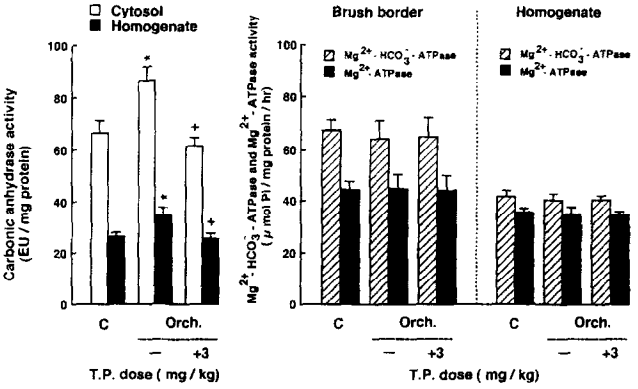

FIG. 2. Effects of TP administration on CA activities (left-hand side) and Mg^{2+}–HCO_3^-–ATPase and Mg^{2+}–ATPase activities (righthand side) of orchiectomized rat kidney. Each column represents the mean ± SD. n = 8. *Statistically significant compared with control (C) ($P < 0.01$). †Statistically significant compared with orchiectomized rat kidney ($P < 0.01$).

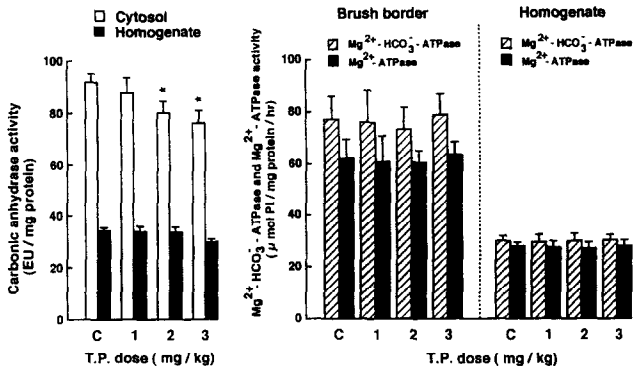

FIG. 3. Effects of TP administration on CA activities (left-hand side) and Mg^{2+}–HCO_3^-–ATPase and Mg^{2+}–ATPase activities (righthand side) of normal female rat kidney. Each column represents the mean ± SD. $n = 7$. *Statistically significant compared with control (C) ($P < 0.05$).

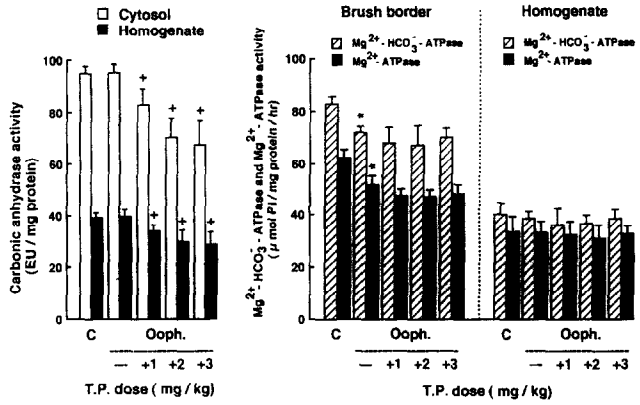

FIG. 4. Effects of TP administration on CA activities (left-hand side) and Mg^{2+}–HCO_3^-–ATPase and Mg^{2+}–ATPase activities (righthand side) of oophorectomized rat kidney. Each column represents the mean ± SD. $n = 6$. *Statistically significant compared with control (C) ($P < 0.05$). †Statistically significant compared with oophorectomized rat kidney ($P < 0.01$).

mogenate were increased after orchiectomy, and these activities were returned to normal levels after TP replacement. The following negative correlation was observed between cytosol CA activity (y) and serum T concentration (nM/l, x):

$$y = -0.906x + 83.793, \quad r = -0.867, n = 24,$$

$$P < 0.01.$$

On the other hand, Mg^{2+}–HCO_3^-–ATPase and Mg^{2+}–ATPase activities in brush border and homogenate (righthand side) did not change in any case.

As shown in Table 1, orchiectomy decreased prostate weight and serum concentrations of T, aldosterone and cortisol-like material (cortisol), and these decreases were recovered to normal or near normal levels after TP replacement. On the other hand, adrenal weight was increased to ~145% of control values after orchiectomy, which was decreased toward the normal values after TP administration. Body weight was not changed in any case (data not shown).

Effects of the Administration of TP to Normal Female Rats

As shown in Fig. 3, CA activities (lefthand side) in cytosol was only decreased after TP administration in a dose-dependent manner. The following negative correlation was observed between cytosol CA activity (y) and serum T concentration (nM/l, x):

$$y = -0.650x + 90.734, \quad r = -0.798, n = 28,$$

$$P < 0.01.$$

On the other hand, Mg^{2+}–HCO_3^-–ATPase and Mg^{2+}–ATPase activities in brush border and homogenate (righthand side) did not change after TP administration.

As shown in Table 1, uterus weight was increased by the administration of 3 mg/kg TP. Serum concentration of T was increased after TP administration in a dose-dependent manner. Body weight was not changed in any case (data not shown).

Effects of the Administration of TP to Oophorectomized Rats

Figure 4 shows the effects of oophorectomy and TP administration (1–3 mg/kg BW/day for 7 days from 1 week after operation). CA activities (lefthand side) in cytosol and homogenate were not affected after oophorectomy; however, TP administration decreased the enzyme activities in a dose-dependent manner. The following correlation was observed between cytosol CA activity (y) and serum T concentration (nM/l, x):

$$y = -1.624x + 93.128, \quad r = -0.850, n = 30,$$

$$P < 0.01.$$

Mg^{2+}–HCO_3^-–ATPase and Mg^{2+}–ATPase activities (righthand side) were decreased after oophorecomy; however, TP administration did not affect these enzyme activities.

In Table 1, uterus weight was decreased after oophorectomy, and TP administration increased its weight in a dose-dependent manner. These increases in uterus weight may be due to the conversion of T to E_2 by aromatase.

DISCUSSION

The main purpose of these experiments was to elucidate whether T and E_2 had a different effect on kidney CA and Mg^{2+}–HCO_3^-–ATPase activities between males and females.

We have previously shown (55) that E_2 increased the kidney CA and Mg^{2+}–HCO_3^-–ATPase activities in normal

TABLE 2. Comparison of changes in kidney enzyme activities after the administration of TP and estradiol dipropionate (EP) to rats

Experimental condition	Relative activity	
	CA	Mg^{2+}–HCO_3^-–ATPase
Normal male control	100	100
Normal male + TP (3 mg/kg/d × 7)	67	98
Orchiectomized (after 2 wk)	131	95
Orchiectomized + TP (3 mg/kg/d × 7)	93	97
Normal female control	100	100
Normal female + TP (3 mg/kg/d × 7)	82	102
Oophorectomized (after 2 wk)	100	87
Oophorectomized + TP (3 mg/kg/d × 7)	71	84
Normal male control	100	100
Normal male + EP (100 μg/kg/d × 7)	149	215
Orchiectomized (after 3 wk)	118	99
Orchiectomized + EP (100 μg/kg/d × 7)	142	182
Normal female control	100	100
Normal female + EP (100 μg/kg/d × 7)	97	143
Oophorectomized (after 3 wk)	97	84
Oophorectomized + EP (200 μg/kg/d × 7)	103	131

Enzyme activities in normal control were expressed as 100. Each value represents the mean from five to eight animals.

and orchiectomized male rats. Further, E_2 also increased brush border Mg^{2+}–HCO_3^-–ATPase activity alone in normal and oophorectomized female rats; there was a sex difference between males and females in E_2 effects.

In Table 2, the effects of T and E_2 on cytosol CA and brush border Mg^{2+}–HCO_3^-–ATPase activities in rat kidney were summarized. Testosterone decreased CA activity in four animal conditions, and E_2 conversely showed an activating effect on CA activity of male rats. Presumably, T has an inhibitory effect on the synthesis of kidney CA; on the other hand, T had no effect on brush border Mg^{2+}–HCO_3^-–ATPase activity in all animal conditions different from E_2.

Furthermore, because activation of CA and brush border Mg^{2+}–HCO_3^-–ATPase by E_2 in male rat kidney was inhibited by the combined administration of actinomycin D or cycloheximide (56), increases in both enzyme activities by E_2 may be related to the synthesis of mRNA and enzyme proteins.

Whether the activation or inhibition of kidney CA by E_2 and T is related to the changes in electrolyte metabolism induced by each hormone is an interesting matter. It is well known that sodium retention occurs in women during pregnancy (9) and after administration of E_2 to adrenalectomized rats (12). Crane and Harris (10) reported that E_2 administration to humans (men and women) increased the plasma renin activity and aldosterone secretion. On the other hand, Johnson *et al.* (27,28) reported with normal and adrenalectomized dogs that Na retention induced by E_2 administration was independent of an action to increase adrenal steroids secretion. Because kidney CA is related to Na reabsorption in renal tubule through Na^+–H^+ exchange process, activation of CA by E_2 may induce the Na retention. Further, if the aldosterone secretion is accelerated by E_2 administration, Na retention is further increased by Na^+–K^+–ATPase, because Na^+–K^+–ATPase in basolateral membrane is assumed to be the target enzyme of aldosterone and activation of this enzyme accelerates the Na reabsorption in renal tubule of rats (41). Despite the effect of E_2 on electrolyte metabolism reported in humans or animals, similar reports on T action are almost nothing as far as we refer. However, inactivation of kidney CA by T seems to be proper, because in general T and E_2 act antagonistically on the physiological functions in living body. Fundamental experiment on electrolyte metabolism by T should be needed to clarify the mechanism of reverse phenomena on kidney CA by both sex steroids.

The similar experiments of the present study have been reported recently on rat liver CA. Hepatic cytosol CA-II isozyme concentration in male rats was much lower compared with that in female rats, and CA-II concentration was increased after orchiectomy and approached to normal female rat levels. CA-II concentration in orchiectomized rat liver further increased over normal female rat levels by the treatment with stilbesterol *in vivo* (24). In the present experiments, we also observed that the specific activity of kidney cytosol CA in male rats was lower than that of females and orchiectomy increased CA activity to female levels (Fig. 2). In contrast, Shiels *et al.* (47) reported that another hepatic CA isozyme, CA-III, concentration in male rats was 30-fold higher than that in the females and that the main hormone that regulated these sexual differences in CA-III concentration was T. In addition, studies that growth hormone controlled the sexually differentiated liver metabolism of drugs and steroids (25,26,42,43) reported that growth hormone was the main hormone for the above sexual difference in CA-II and CA-III concentrations. However, role of growth hormone to maintain the difference in kidney CA activity between males and females has not been elucidated. Although

the physiological role of CA in the liver and kidney may be different, that the reaction patterns of kidney CA activity to orchiectomy and E_2 administration in our studies are similar to those reported with hepatic CA-II concentration by Jeffery *et al.* (24) is of great interest.

Recently, Härkönen *et al.* (21) reported that the CA-II concentration in rat lateral prostate was decreased after orchiectomy and T replacement recovered its enzyme concentration to normal levels. On the other hand, CA-II concentration and mRNA levels in dorsal prostate were conversely increased after orchiectomy and T administration recovered its enzyme concentration and mRNA levels to near normal levels, indicating that the effects of castration and T replacement on CA were reversed between lateral and dorsal prostate. Although the difference in physiological significance of CA-II in prostate and kidney is not clear at present, induction of CA-II in dorsal prostate by orchiectomy and their recovery after T administration is quite similar to the case of male rat kidney CA.

The correlation between adrenal and testicular functions has been already reported. Kitay (32) has shown that the orchiectomy induced the increase in adrenal weight and the decrease in corticosterone secretion from adrenals in male rats, and further the replacement with T returned them to normal or near normal levels. Kubota and Suzuki (35) also observed the adrenocortical hypertrophy after castration and its return to the normal after TP administration (5 mg/kg BW/d for 2 weeks) in adult male rats. In the present experiments, adrenal weight was increased and serum concentrations of aldosterone and cortisol-like material were decreased after orchiectomy, and replacement with TP recovered these values to normal or near normal levels. However, because of the no essential effect of adrenalectomy on kidney CA activity (52), decreases in serum adrenocorticoids after orchiectomy seem to have no direct effect on the elevation of kidney CA activity by orchiectomy (Table 1 and Fig. 2).

The results in the present experiment revealed that the male and female sex steroids act antagonistically on kidney CA activity. Because the physiological significance of CA in the electrolyte metabolism in the kidney has been documented, above phenomena may show the effect of T and E_2 on the maintenance of electrolyte balance in the living body. However, because recent studies aim to clarify the effect of androgens in molecular levels (5,8,23), studies on purification of CA isozymes, on synthesis of mRNA, on the possibility of enzyme protein conformation and the correlation between above matters and electrolyte metabolism should be needed to elucidate the mechanism of action of T on the kidney.

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