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SEXUAL DIFFERENCE AND ORGAN SPECIFICITY OF THE EFFECT OF ESTRADIOL ON CARBONIC ANHYDRASE AND Mg^{2+}-HCO_3^--ATPase ACTIVITIES ISOLATED FROM DUODENAL MUCOSA AND KIDNEY CORTEX OF MALE AND FEMALE RATS: PRELIMINARY STUDY WITH CRUDE ENZYME SAMPLES

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Summary—Effects of the s.c. administration of various doses of estradiol propionate (E.P.; 25–500 μg/kg) on the activities of carbonic anhydrase (CA), Mg^{2+}-dependent ATPase and Mg^{2+}-dependent, HCO_3^--stimulated ATPase (Mg^{2+}-HCO_3^--ATPase) in rat duodenal mucosa and kidney cortex, and on body weight, organ weight and serum concentrations of testosterone and estradiol-17β, were examined in adult male, female, testectomized and ovariectomized rats. In normal male rats, activities of cytosol CA and brush border Mg^{2+}-HCO_3^--ATPase in the kidney were increased in a dose-dependent manner and reached 1.6- and 2-fold of controls, respectively, after consecutive administration (daily for 7 days) of 500 μg E.P. with no changes in either enzyme activities in duodenal mucosa. The positive correlations ($P < 0.01$) were observed by linear regression analysis between serum concentration of estradiol-17β and kidney cytosol CA or kidney brush border Mg^{2+}-HCO_3^--ATPase activities. In normal female rats, activities of cytosol CA and brush border Mg^{2+}-HCO_3^--ATPase in the duodenal mucosa, and brush border Mg^{2+}-HCO_3^--ATPase activity in the kidney were increased by E.P. administration (100 and 500 μg/kg, daily for 7 days), however, kidney cytosol CA activity did not change by any dosage. Behavior of a part of both enzymes to E.P. in testectomized rats was altered almost in the same way to that observed in normal female rats and *vice versa* in ovariectomized rats. Body weight was decreased, in general, by consecutive administration of E.P. in a dose-dependent manner, and kidney weight was increased by E.P. in both male and female rats.

INTRODUCTION

Carbonic anhydrase (CA) was first isolated from red blood cells [1]. It is a zinc metalloenzyme which catalyses the reversible hydration of carbon dioxide, and also functions in the transfer of protons and bicarbonate ions in secretory epithelium [2, 3]. Recently, the presence of several CA isoenzymes, CA-I, CA-II and CA-III, identified by immunohistochemical method or purification procedures, has been reported in the cytoplasm of various tissues, including blood, gastric mucosa, intestinal mucosa, liver and kidney [4–6], however, their specific physiological roles on ion transport have not been fully elucidated.

Another characteristic of CA is that the enzyme activities or concentrations in various organs are influenced by several hormones and, at the same time, the effects of hormones seem to have an organ specificity. Effects of female sex hormones on CA activity of uterine endometrium have already been examined and it has been observed that there are species differences between mice and rats. Male and female sex hormones also affect CA activities or isoenzyme concentrations in rat liver [7–9]. Despite these studies, the physiological significance of sex hormones on CA and the effect of estradiol (E_2) on CA activity in rat duodenal mucosa and kidney cortex have not been investigated.

Mg^{2+}-dependent, HCO_3^--stimulated ATPase (Mg^{2+}-HCO_3^--ATPase) has been found in

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various organs, although there are many unsolved problems concerning the subcellular localization and physiological role of this enzyme. Mg^{2+}-HCO_3^--ATPase in the brush border of rat small intestinal mucosa was first reported by Humphreys and Chou [10] and assumed to be a type of alkaline phosphatase [11]. Although the physiological functions of this enzyme in the duodenal mucosa have not been fully established, Gerencser and Lee [12] suggested that its involvement in the membrane transport of HCO_3^- is through the HCO_3^--Cl^- exchange process. Mg^{2+}-HCO_3^--ATPase activity in the brush border of rat kidney has also been reported [13, 14], however, its physiological roles are now under discussion. To date, the effects of sex hormones on the brush border Mg^{2+}-HCO_3^--ATPase in rat intestinal mucosa and kidney have not been investigated.

We reported recently that the specific activities of cytosol CA and brush border Mg^{2+}-HCO_3^--ATPase in rat duodenal mucosa are sensitive to adrenalectomy [15] or hyper- and hypothyroidism [16]. In contrast, both enzyme activities in the kidney did not change under the same hormonal unbalance. In the present experiment, the effects of E_2 on CA and Mg^{2+}-HCO_3^--ATPase activities from male and female rats were examined to clarify the following: (1) whether the changes in above enzyme activities in the duodenal mucosa are specific to adrenal or thyroidal disorders; (2) whether the changes in the activities of CA and Mg^{2+}-HCO_3^--ATPase under hormonal disorders are only restricted to duodenal mucosa; and (3) whether the changes in enzyme activity induced by the administration of E_2 are also found in the kidney.

EXPERIMENTAL

Animals

Laboratory bred male and female Wistar rats (7–8 weeks old and weighing 200–250 g in males and 150–180 g in females at the start of the experiment) were used. Testectomy and ovariectomy were performed under pentobarbital anaesthesia (Nembutal sodium, Abbott Laboratories, North Chicago, IL, U.S.A.; 20 mg/kg body weight, i.p.). Operated and sham-operated or normal control animals were maintained on a commercial solid diet with tap water available *ad libitum*. All animals were housed at room temperature (24°C) on a 12 h light–darkness cycle.

Chemicals

An oily injection of estradiol propionate (E.P.; Ovahormone, Teikokuzoki Pharmaceutical Co., Tokyo, Japan), diluted with sesame oil to desired concentrations, was injected s.c. in 0.1 ml solvent/100 g body weight. Sham-operated or normal control animals were treated with the same volume of sesame oil. Na_2ATP (Boehringer, Mannheim, Fed. Rep. Germany) was dissolved in distilled water and adjusted to pH 8 with Tris-base. Other chemicals used for the preparation of enzyme samples and for the assay of their enzyme activities were all reagent grade.

Sample preparation procedures

All animals were killed between 10.00 and 15.00 h on the day of the experiment. Rats were anaesthetized with pentobarbital (30 mg/kg body weight, i.p.) and blood (about 4 ml) was taken from the femoral artery into glass testtubes and left for several hours at room temperature. After coagulation of the blood, serum was separated by centrifugation at 1870 ***g*** for 10 min. Immediately after the collection of the blood sample, the abdomen was opened and the whole body was perfused with heparinized saline through the portal vein and aorta. The duodenal portion, approx. 10 cm in length of the intestine, was severed from the pylorus. The duodenal lumen was flushed with cold saline and duodenal mucosa was scraped off with a glass slide and weighed. The kidneys were removed, decapsulated and pieces of the cortex were washed with cold saline. Adrenal glands, prostate and uterus were 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 that described by Schmitz *et al.* [17] and Malathi *et al.* [18]. In brief, duodenal mucosa and kidney cortex were homogenized in a Potter–Elvehjem type glass Teflon homogenizer with 10 ml ice-cold 50 mmol D-mannitol–2 mmol Tris–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 mmol and the mixture was gently stirred in iced water 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.

Enzyme assay

Carbonic anhydrase activities in the homogenate, brush border and supernatant fractions were assayed by a colorimetric method [19]. Enzyme activity was calculated according to the expression: enzyme activity unit = $(T_0\text{-}T)/T$ 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+}-ATPase and Mg^{2+}-HCO_3^--ATPase activities in homogenate and brush border were assayed as described previously [20]. Aliquots of samples were placed in test-tubes (15 × 100 mm) with 1.0 mmol $MgCl_2$, 50 mmol $NaHCO_3$, 3 mmol ATP and 50 mmol 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 placed in iced water and the reaction was stopped by adding 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) was determined by the method of Allen [21] as modified by Nakamura [22]. Enzyme activity was expressed as μmol Pi/mg protein per h. Mg^{2+}-ATPase activity was assayed in the absence of $NaHCO_3$.

Hormone assay

Serum hormone concentrations were assayed by radioimmunoassay (RIA) using the following commercial assay kits: E_2 direct RIA-Kit using a double-antibody method (SORIN BIOMEDICA STA, Saluggia, VC, Italy) and testosterone RIA-Kit using a double-antibody method (Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) For E_2, this assay has a minimum 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. For testosterone, this assay has a minimum 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.

Protein assay

Protein in the enzyme samples was determined by the method of Lowry *et al.* [23] with bovine serum albumin as a standard.

Statistical analysis

Student's unpaired *t*-test was used to assess the statistical significance of differences in values. Linear regression analysis was carried out using a programmed calculator.

RESULTS

Effects of the administration of E.P. to normal male rats

Figure 1 shows the effects of E.P. at doses of 25–500 μg/kg body weight per day s.c. for 7 days on enzyme activities.

In the duodenal mucosa (left side), activities of CA in the homogenate and cytosol did not change by any dosage [Fig. 1(a)]. Both ATPase activities in the homogenate and brush border also did not change after the administration of E.P. [Fig. 1(b) and (c)].

In the kidney (right side), however, activities of CA in the homogenate and cytosol were increased and reached about 1.6-fold of control value by the administration of 500 μg E.P./kg in both fractions [Fig. 1(d)]. Mg^{2+}-ATPase and Mg^{2+}-HCO_3^--ATPase activities in the homogenate and brush border were increased by E.P. with increasing dosage and reached about 2-fold of control by the administration of 500 μg E.P./kg in both fractions [Fig. 1(e) and (f)].

Table 1 shows the changes in body weight gain or loss for 1 week, adrenal, prostate, duodenal mucosa and kidney weights at autopsy, and serum concentrations of testosterone and E_2. Body weight gain, prostate weight and serum concentration of testosterone were decreased by the administration of E.P., while adrenal weight, kidney weight and serum concentration of E_2 were increased after the administration of E.P. with no changes in duodenal mucosal weight.

Interrelation between serum E_2 concentration and kidney enzyme activity

From the data presented in Fig. 1 and Table 1, interrelation between serum concentration of E_2 and activities of cytosol CA or

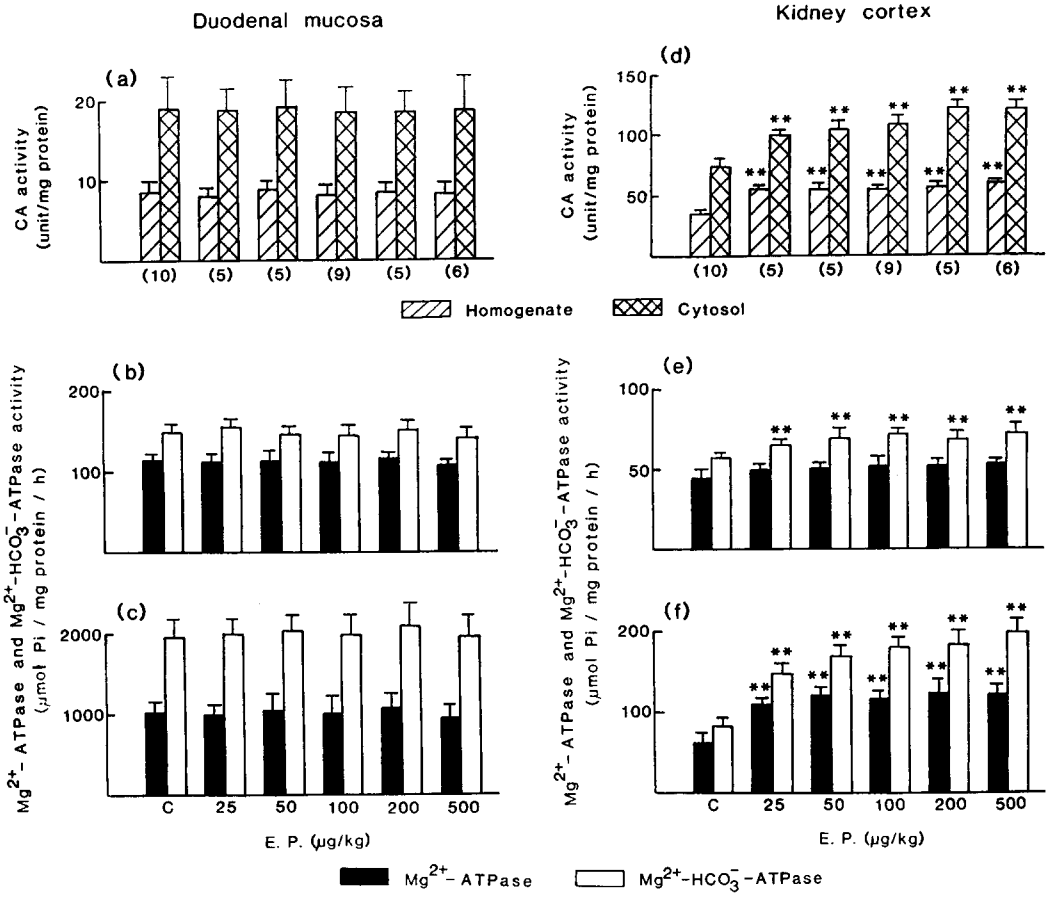

Fig. 1. Effects of E.P. administration to normal male rats. Figures on the lefthand side indicate the CA activities in the homogenate and cytosol (a), ATPase activities in the homogenate (b) and brush border (c) of duodenal mucosa, respectively. Figures on the righthand side indicate the CA activities in the homogenate and cytosol (d) and ATPase activities in the homogenate (e) and brush border (f) of kidney cortex, respectively. Each column represents the mean ± SD and numbers of animals are given in parentheses. $^{**}P < 0.01$ compared with controls (C).

brush border Mg^{2+}-HCO_3^--ATPase in the kidney was calculated by linear regression analysis. As shown in Fig. 2, increases in the activities of cytosol CA [Fig. 2(a)] and brush border Mg^{2+}-HCO_3^--ATPase [Fig. 2(b)] were parallel with the increases in serum concentration of E_2 and positive correlations ($P < 0.01$) were observed between them.

Effects of the administration of E.P. to testectomized rats

Figure 3 shows the effects of the administration of E.P. at doses of 100 and 500 μg/kg body weight per day s.c. for 7 days from 2 weeks after testectomy on enzyme activities.

In the duodenal mucosa (left side), activities of CA in homogenate and cytosol were

Table 1. Effects of E.P. administration on various parameters in male rats

Dose of E.P. (μg/kg)	B.wt. (g) gain (+) or loss (−)	Adrenal wt. (mg/ 100 g B.wt.)	Prostate wt. (mg/ 100 g B.wt.)	Duo. m. wt. (mg/ 100 g B.wt.)	Kidney wt. (mg/ 100 g B.wt.)	Serum concentration (nmol/l)	
						Testosterone	E_2
Control (10)	+30.5 ± 6.4	18.9 ± 1.7	239 ± 13	135 ± 16	707 ± 44	11.36 ± 4.02	0.039 ± 0.019
25 (5)	+5.0 ± 5.1**	25.8 ± 4.4**	156 ± 20**	126 ± 14	805 ± 101**	1.80 ± 0.88**	0.352 ± 0.060**
50 (5)	+2.6 ± 2.0**	26.8 ± 1.1**	140 ± 25**	125 ± 14	828 ± 62**	1.55 ± 0.65**	0.778 ± 0.076**
100 (9)	−11.2 ± 8.2**	26.3 ± 2.4**	135 ± 23**	135 ± 13	807 ± 45**	1.05 ± 0.50**	1.484 ± 0.412**
200 (5)	−10.0 ± 8.2**	26.6 ± 3.6**	135 ± 20**	125 ± 12	836 ± 58**	0.47 ± 0.29**	2.776 ± 0.374**
500 (6)	−16.8 ± 4.0**	30.9 ± 1.1**	122 ± 25**	122 ± 14	860 ± 62**	ND	4.486 ± 0.541**

Values are means ± SD, numbers of animals are given in parentheses. B.wt.; body weight. Duo. m.; duodenal mucosa. ND; not detectable.
$^{**}P < 0.01$ compared with control.

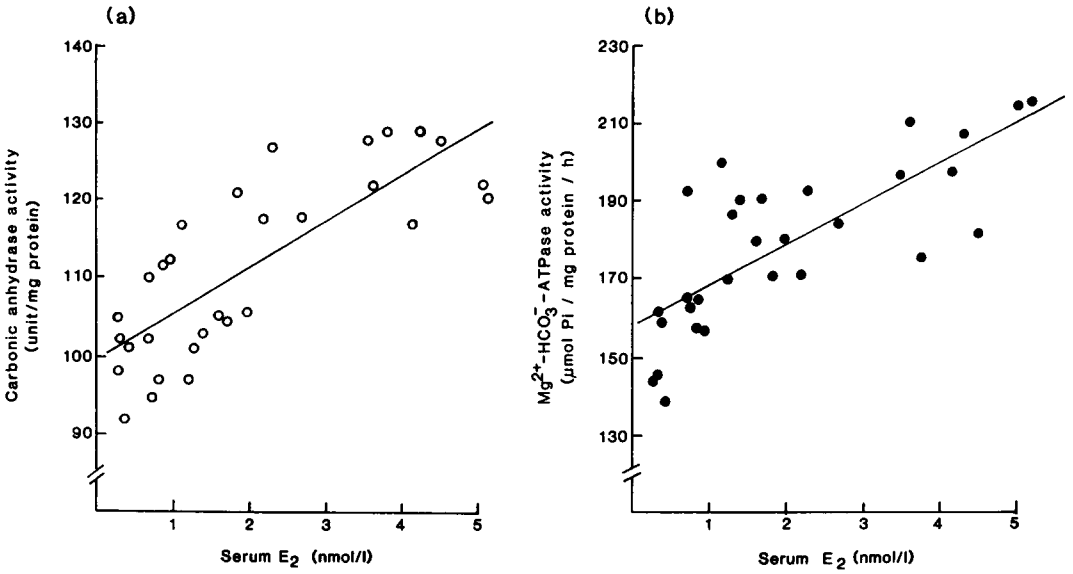

Fig. 2. Correlations between (a) serum concentration of E_2 and carbonic anhydrase activity of kidney cytosol and (b) serum concentration of E_2 and Mg^{2+}-HCO_3^--ATPase activity of kidney brush border in E.P.-treated male rats. In (a) $Y = 99.827 + 5.877X$, $r = 0.7888$, $n = 30$, $P < 0.01$, in (b) $Y = 158.313 + 10.442X$, $r = 0.7620$, $n = 30$, $P < 0.01$.

increased after testectomy and administration of E.P. restored the increased enzyme activities to normal levels [Fig. 3(a)]. Both ATPase activities in the homogenate were not changed after testectomy and the administration of E.P. [Fig. 3(b)]. Mg^{2+}-ATPase and Mg^{2+}-HCO_3^--ATPase activities in the brush border did not change after testectomy, however, both ATPase activities increased by the administration of E.P. [Fig. 3(c)].

In the kidney (right side), activities of CA in the homogenate and cytosol were increased after testectomy and the administraton of E.P. further increased the enzyme activities in both fractions [Fig. 3(d)]. Activities of Mg^{2+}-ATPase and Mg^{2+}-HCO_3^--ATPase in the homogenate and brush border did not change after testectomy, however, the administration of E.P. increased both ATPase activities in both fractions [Fig. 3(e) and (f)].

Table 2 shows the effects of testectomy and the administration of E.P. on various parameters. Body weight gain was not changed by testectomy, while the weight was significantly decreased by the administration of E.P. for 1 week. Adrenal weight, kidney weight and serum concentration of E_2 were increased by the administration of E.P., while prostate weight and serum concentration of testosterone was decreased by E.P. treatment with no changes in prostate and duodenal mucosal weights.

Effects of the administration of E.P. to normal female rats

Prior to this experiment, we examined the relationships between serum concentration of E_2 and activities of CA or Mg^{2+}-HCO_3^--ATPase, between serum concentration of E_2 and uterine weight, and between uterine weight and both enzyme activities in the duodenal mucosa and kidney of normal female rats, because the estrus cycle might have some effect on enzyme activity. However, no statistically positive correlations were observed between them. Therefore, we used the rats at random as control in this experiment.

Figure 4 shows the effects of E.P. at doses of 100 and 500 μg/kg body weight per day s.c. for 7 days on enzyme activities.

In the duodenal mucosa (left side), activities of CA in homogenate and cytosol were increased after the administration of E.P. [Fig. 4(a)]. Activities of Mg^{2+}-ATPase and Mg^{2+}-HCO_3^--ATPase in the homogenate and brush border were also increased after the administration of E.P., however, a dose-dependent relationship was not clear [Fig. 4(b) and (c)].

In the kidney (right side), activities of CA in the homogenate and cytosol were not changed after the administration of E.P. [Fig. 4(d)]. Activities of Mg^{2+}-ATPase and Mg^{2+}-HCO_3^--ATPase in the homogenate were not changed by E.P. [Fig. 4(e)], while both ATPase activities in

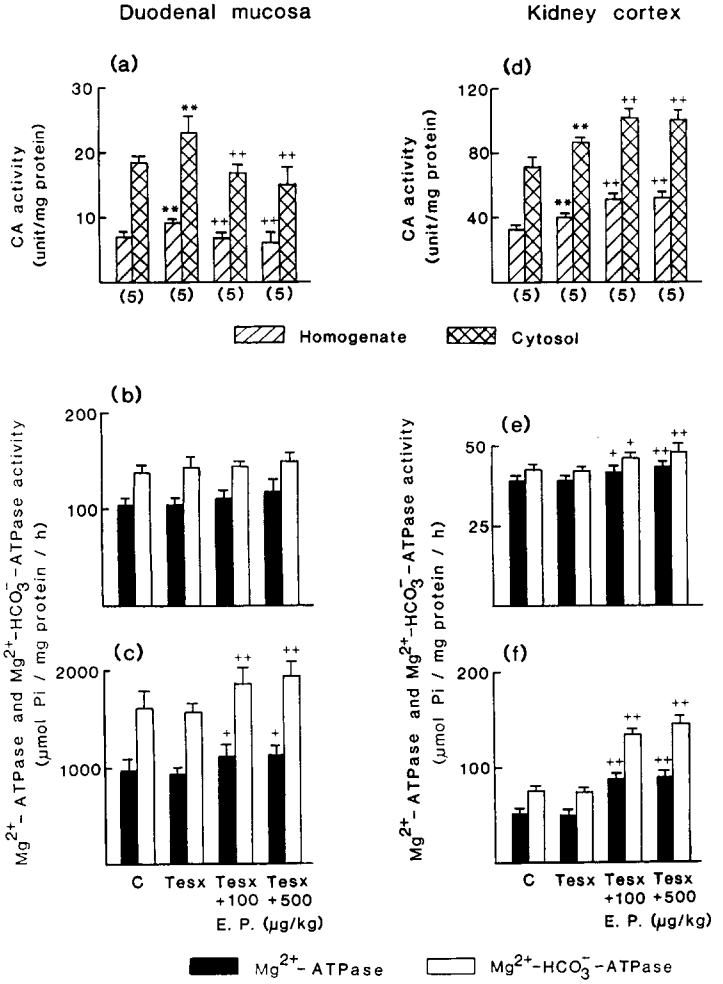

Fig. 3. Effects of E.P. administration to testectomized (Tesx) rats. Figures on the lefthand side indicate the CA activities in the homogenate and cytosol (a), ATPase activities in the homogenate (b) and brush border (c) of duodenal mucosa, respectively. Figures on the righthand side indicate the CA activities in the homogenate and cytosol (d), ATPase activities in the homogenate (e) and brush border (f) of kidney cortex, respectively. Each column represents the mean ± SD and numbers of animals are given in parentheses. $^{**}P < 0.01$ compared with controls (C). $^{+}P < 0.05$, $^{++}P < 0.01$ compared with Tesx group.

the brush border were increased after the administration of E.P., but a dose-dependent relationship was not observed [Fig. 4(f)].

Table 3 shows the effects of the administration of E.P. on various parameters. Body weight gain was significantly inhibited by E.P. administration for 1 week in a dose-dependent manner. Uterus weight, kidney weight and serum concentration of E_2 were increased by E.P. in a dose-dependent manner, while duodenal mucosal weight was not changed.

Table 2. Effects of testectomy (Tesx) and E.P. administration on various parameters in male rats

Group	B.wt. (g) gain (+) or loss (−) start–2 weeks	B.wt. (g) gain (+) or loss (−) 2 weeks–3 weeks	Adrenal wt. (mg/100 g B.wt.)	Prostate wt. (mg/100 g B.wt.)	Duo. m. wt. (mg/100 g B.wt.)	Kidney wt. (mg/100 g B.wt.)	Serum concentration (nmol/l) Testosterone	Serum concentration (nmol/l) E_2
Sham-op. control (5)	+38.2 ± 7.8	+13.2 ± 6.3	14.7 ± 1.8	238 ± 34	114 ± 13	643 ± 17	14.14 ± 3.67	0.092 ± 0.058
Tesx 3 weeks (5)	+32.8 ± 9.6	+20.4 ± 4.9	16.7 ± 1.2	16.3 ± 3.5**	114 ± 10	592 ± 23**	0.81 ± 0.06**	0.073 ± 0.017
Tesx 2 weeks + E.P. 100 μg (5)	+29.2 ± 5.9	−8.4 ± 6.4‡	23.7 ± 2.1‡	15.7 ± 4.8	121 ± 14	741 ± 67‡	0.84 ± 0.15	1.612 ± 0.127‡
Tesx 2 weeks + E.P. 500 μg (5)	+35.0 ± 3.7	−15.2 ± 8.7‡	27.2 ± 1.7‡	13.8 ± 3.5	120 ± 17	757 ± 46‡	0.86 ± 0.33	4.333 ± 0.690‡

Values are means ± SD, numbers of animals are given in parentheses. B.wt.; body weight. Duo. m.; duodenal mucosa. $^{**}P < 0.01$ compared with control. $‡P < 0.01$ compared with Tesx 3 weeks group.

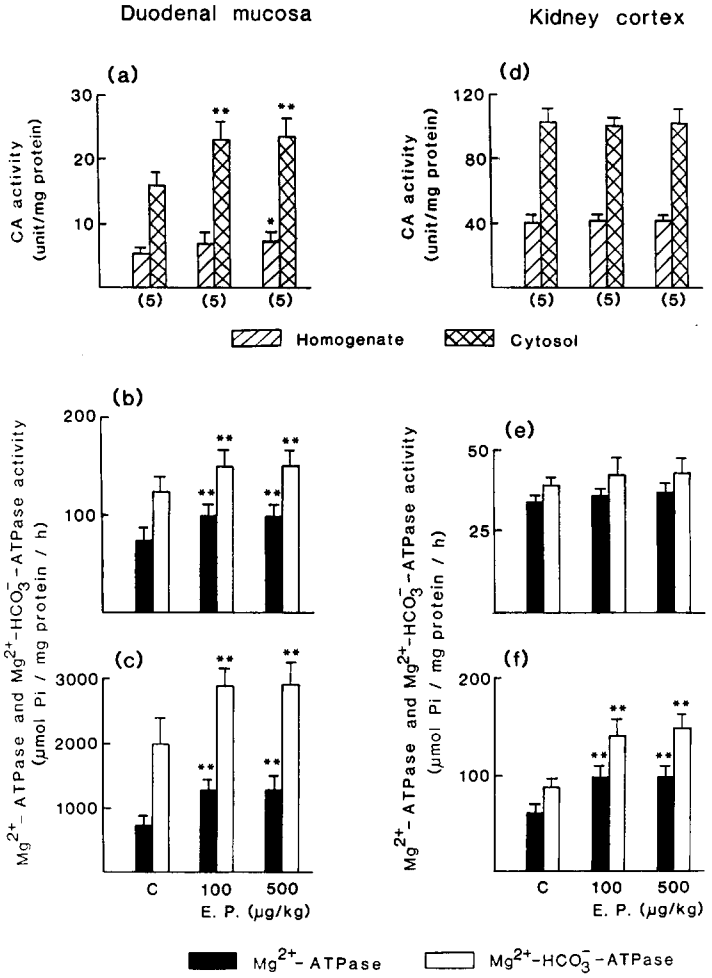

Fig. 4. Effects of E.P. administration to normal female rats. Figures on the lefthand side indicate the CA activities in the homogenate and cytosol (a), ATPase activities in the homogenate (b) and brush border (c) of duodenal mucosa, respectively. Figures on the righthand side indicate the CA activities in the homogenate and cytosol (d), ATPase activities in the homogenate (e) and brush border (f) of kidney cortex, respectively. Each column represents the mean ± SD and numbers of animals are given in parentheses. $*P < 0.05$, $**P < 0.01$ compared with controls (C).

Effects of the administration of E.P. to ovariectomized rats

Figure 5 shows the effects of the administration of E.P., 200 μg/kg body weight per day s.c. for 7 days from 2 weeks after ovariectomy, on enzyme activities.

In the duodenal mucosa (left side), CA activities in the homogenate and cytosol did not change after ovariectomy and replacement with E.P. [Fig. 5(a)]. Mg^{2+}-ATPase and Mg^{2+}-HCO_3^--ATPase activities in the homogenate and brush border also did not change after ovariectomy and the administration of E.P. [Fig. 5(b) and (c)].

In the kidney (right side), CA activities in the homogenate and cytosol and Mg^{2+}-ATPase activities in the homogenate were unchanged after ovariectomy and E.P. administration [Fig. 5(d)], while Mg^{2+}-HCO_3^--ATPase activity in the

Table 3. Effects of E.P. administration on various parameters in female rats

Dose of E.P. (μg/kg)	B.wt. (g) gain for 1 week	Adrenal wt. (mg/ 100 g B.wt.)	Uterus wt. (mg/ 100 g B.wt.)	Duo. m. wt. (mg/ 100 g B.wt.)	Kidney wt. (mg/ 100 g B.wt.)	Serum E_2 concentration (nmol/l)
Control (5)	21.0 ± 3.6	36.7 ± 3.3	211 ± 29	185 ± 13	763 ± 66	0.190 ± 0.033
100 (5)	11.0 ± 1.2**	36.5 ± 2.2	298 ± 16**	172 ± 19	886 ± 27**	1.009 ± 0.194**
500 (5)	5.0 ± 3.8**	34.0 ± 4.3	341 ± 55**	181 ± 28	873 ± 58*	3.847 ± 0.550**

Values are means ± SD, numbers of animals are given in parentheses. B.wt.; body weight. Duo. m.; duodenal mucosa. $*P < 0.05$, $**P < 0.01$ compared with control.

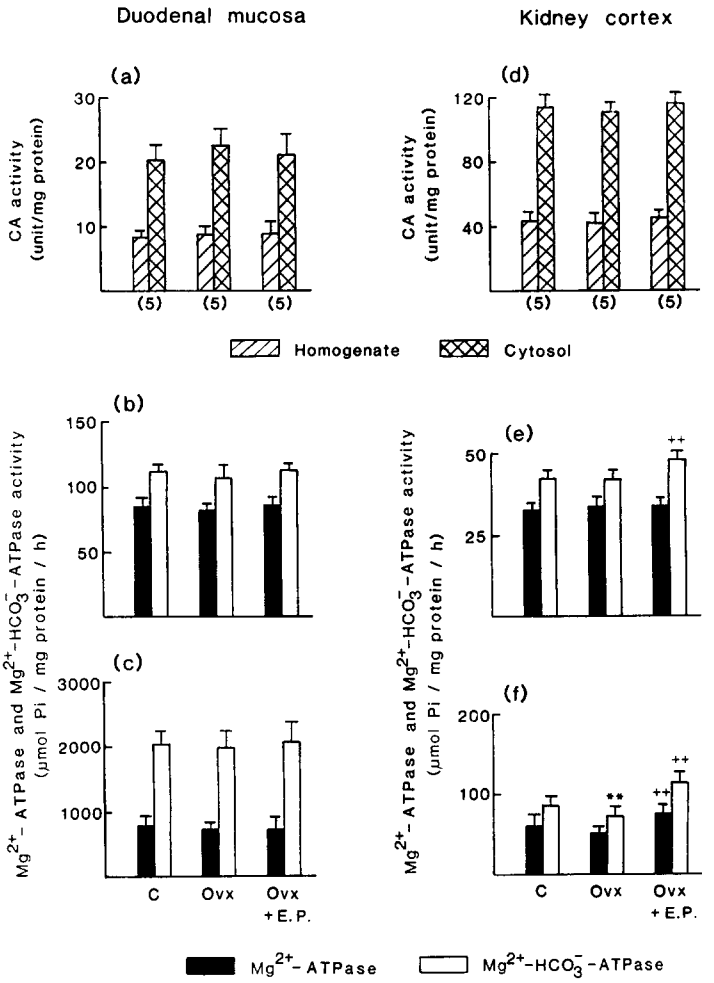

Fig. 5. Effects of E.P. administration to ovariectomized (Ovx) rats. Figures on the lefthand side indicate the CA activities in the homogenate and cytosol (a), ATPase activities in the homogenate (b) and brush border (c) of duodenal mucosa, respectively. Figures on the righthand side indicate the CA activities in the homogenate and cytosol (d), ATPase activities in the homogenate (e) and brush border (f) of kidney cortex, respectively. Each column represents the mean ± SD and numbers of animals are given in parentheses. $^{**}P < 0.01$ compared with controls (C), $^{++}P < 0.01$ compared with Ovx group.

homogenate was only increased by E.P. [Fig. 5(e)]. Further, Mg^{2+}-HCO_3^--ATPase activity in the brush border was only decreased after ovariectomy, and E.P. administration significantly increased both Mg^{2+}-ATPase and Mg^{2+}-HCO_3^--ATPase activities [Fig. 5(f)].

Table 4 shows the effects of ovariectomy and the administration of E.P. on various parameters. Ovariectomy had a tendency to increase the body weight gain, however, administration of E.P. inhibited its gain. Adrenal weight was not changed by ovariectomy, while its weight was increased by the administration of E.P. Uterus weight was significantly decreased by ovariectomy and administration of E.P. restored its weight to a normal level. Kidney weight was increased by the administration of E.P. with no changes in

Table 4. Effects of ovariectomy (Ovx) and E.P. administration on various parameters in female rats

Group	B.wt. (g) gain (+) or loss (−) start–2 weeks	B.wt. (g) gain (+) or loss (−) 2 weeks–3 weeks	Adrenal wt. (mg/100 g B.wt.)	Uterus wt. (mg/100 g B.wt.)	Duo. m. wt. (mg/100 g B.wt.)	Kidney wt. (mg/100 g B.wt.)	Serum E_2 concentration (nmol/l)
Sham-op. control (5)	+30.4 ± 5.5	+15.0 ± 4.0	35.2 ± 5.4	201 ± 56	146 ± 14	674 ± 31	0.248 ± 0.058
Ovx 3 weeks (5)	+41.8 ± 8.2	+18.8 ± 4.7	32.4 ± 4.9	44 ± 5**	148 ± 14	643 ± 28	0.094 ± 0.016**
Ovx 2 weeks + E.P. 200 μg/kg (5)	+38.6 ± 8.1	−8.0 ± 3.6‡	36.9 ± 2.0‡	253 ± 23‡	142 ± 12	726 ± 37‡	1.790 ± 0.422‡

Values are means ± SD, numbers of animals are given in parentheses. B.wt.; body weight. Duo. m.; duodenal mucosa. $^{**}P < 0.01$ compared with control. $‡P < 0.01$ compared with ovariectomized (Ovx) group.

duodenal mucosa weight. Serum E_2 concentration was decreased after ovariectomy and the administration of E.P. significantly increased its concentration.

Effects of E.P. on brush border membrane-bound CA activity

Table 5 shows the effects of E.P. administration on brush border membrane-bound CA activities in duodenal mucosa and kidney cortex of normal male and female rats. E_2 administration significantly increased CA activity in the kidney of male rats with no changes in duodenal mucosa, in contrast, E.P. administration had a tendency to increase duodenal CA activity with no effect on kidney CA activity in female rats.

DISCUSSION

Previous studies revealed that the activities of CA and Mg^{2+}-HCO_3^--ATPase in male rat kidney were not changed after adrenalectomy and administration of adrenocorticoids [15] and after thyroidectomy and administration of thyroxine [16]. In the present experiment, however, activities of cytosol CA and brush border Mg^{2+}-HCO_3^--ATPase in the male rat kidney were increased to about 1.6- and 2-fold of controls by the consecutive administration of 200 or 500 μg E.P./kg with no changes in either enzyme activities in the duodenal mucosa (Fig. 1). These phenomena seem to be the most prominent differences among E_2, adrenocorticoids and thyroid hormone in male rats.

In addition, effects of E.P. on the specific activities of CA and Mg^{2+}-HCO_3^--ATPase from duodenal mucosa also seem to be different between male and female rats. In normal male (Fig. 1) and ovariectomized female rats (Fig. 5), activities of duodenal cytosol CA and brush border Mg^{2+}-HCO_3^--ATPase did not change in many cases after E.P. administration. In contrast, administration of E.P. to normal female rats increased both duodenal CA and Mg^{2+}-HCO_3^--ATPase activities (Fig. 4). Therefore, ovariectomy seems to bring the sensitivity of duodenal two enzyme systems against E.P. toward the male rat type. Testectomy increased kidney CA activity to 120% of normal levels and administration of E.P. further increased its enzyme activity to about 140% of normal (Fig. 3). In contrast, our previous experiment [24] already showed that the elevation of kidney cytosol CA activity in the male rat after testectomy decreased to normal levels by testosterone replacement. Therefore, endogenous testosterone seems to act as an inhibitory factor on the activity of kidney CA and exogeneous estrogen may act as an activating factor for CA in male rat kidney, that is, it can be said that testosterone and E_2 are acting antagonistically on the kidney cytosol CA system in the male rat. According to Wistrand and Wåhlstrand [25] and Dobyan and Bulger [4], rat kidney cytosol has only one type of CA isoenzyme, CA-II, identical to erythrocyte CA-II. Therefore, changes in the kidney cytosol CA activity in the present experiment may be due to the changes in CA-II activity.

Sexual differences in the reaction of CA isoenzymes to sex hormones have already been reported by several investigators in the rat liver. For example, hepatic cytosol CA-II isoenzyme concentration in male rats is much lower compared with that of females and after testectomy CA-II concentration increased and approached the normal female rat levels, CA-II concentration in testectomized rat liver further increased over normal female rat levels by treatment with stilbesterol *in vivo* [9]. In the present experiment, we also observed that the specific activity of kidney cytosol CA in male rats (Fig. 1) is lower (60 ~ 70 U/mg protein) than that in female rats (about 110 U/mg protein) (Fig. 4). In contrast, Shiels *et al.* [26] reported that another hepatic cytosol CA

Table 5. Effects of E.P. administration on brush border membrane-bound CA activities in male and female rats

	Carbonic anhydrase activity/mg protein			
	Male (5)		Female (5)	
Group	Duo. m.	Kidney cortex	Duo. m.	Kidney cortex
Control	1.52 ± 0.27	14.82 ± 1.87	1.16 ± 0.47	23.69 ± 2.01
+E.P. 25 μg/kg	1.70 ± 0.53	20.87 ± 1.31**	—	—
+E.P. 50 μg/kg	1.62 ± 0.56	20.87 ± 1.87**	—	—
+E.P. 100 μg/kg	1.53 ± 0.47	21.88 ± 1.06**	1.33 ± 0.40	23.94 ± 0.70
+E.P. 200 μg/kg	1.43 ± 0.34	22.00 ± 1.65**	—	—
+E.P. 500 μg/kg	1.56 ± 0.52	22.02 ± 1.48**	1.44 ± 0.37	22.96 ± 1.13

E.P. was administered s.c. daily for 7 days. Values are means ± SD, numbers of animals are given in parentheses. Duo. m.; duodenal mucosa. **$P < 0.01$ compared with control.

isoenzyme, CA-III, concentration in male rats is 30-fold higher than that in the females and that the main hormone which regulates these sexual differences in CA-III isoenzyme concentration is testosterone. In addition, Jeffery *et al.* [27] reported that phypophysectomy abolished these sexual differences in liver CA-II and CA-III concentrations of female rats and growth hormone administration to male rats induced both isoenzyme concentration patterns to the female rat type. Although the physiological role of CA in the liver and kidney may be different, the reaction pattern of kidney CA activity to testectomy and E.P. administration was similar to that reported with hepatic CA-II concentration by Jeffery *et al.* [9].

Recently, brush border membrane-bound CA isoenzyme, CA-IV, has been reported in rat kidney [28–30], and Wistrand and Kinne [29] reported that the specific activity of brush border membrane CA is almost the same as that of cytosol CA. As shown in Table 5, effects of E.P. on brush border membrane-bound CA activities of duodenal mucosa and kidney were the same as those observed with cytosol CA of normal male and female rats, however, kidney brush border CA activity was very low compared with that of cytosol CA. The reason for this may be due to the fact that the kidney brush border used in the present experiment has not yet been purified, because this brush border sample contained about 30 protein bands, by SDS–polyacrylamide-gel electrophoresis.

The enzyme isolation techniques used in this experiment are simple and classical. Since the physiological significance of the reaction of kidney CA and/or Mg^{2+}-HCO_3^--ATPase to E_2 is not clear, the study using the specific antisera against each isoenzyme or electrophoretic analysis should be performed to ascertain the interrelationship between enzyme reaction and hormonal effects.

Segal and Koide [31] and Rories and Spelsberg [32] have shown that E_2 finally causes the changes in levels of steady-state specific RNAs and proteins. Therefore, the activation of CA and Mg^{2+}-HCO_3^--ATPase by E.P. in the present experiment may be due to the increase of enzyme protein synthesis, which is shown by the following studies; maximum enzyme activities of alkaline phosphatase and Mg^{2+}-HCO_3^--ATPase isolated from purified male rat kidney brush border by free flow electrophoresis were eluted in the same fraction and further Mg^{2+}-HCO_3^--ATPase is only located in the brush border membrane and is lacking in the basal lateral membrane of the tubular cells [13], consecutive administration of estrogen increased kidney alkaline phosphatase activity in the developing or adult male and female rats [33]. In contrast, kidney alkaline phosphatase activity decreased after castration in female rats [34]. An increase in alkaline phosphatase activity induced by estrogen administration was inhibited by the combined administration of actinomycin D [35]. We also observed that the activations of kidney CA and Mg^{2+}-HCO_3^--ATPase by E.P. administration to male rats were inhibited by the combined administrations of actinomycin D and cycloheximide, RNA and protein synthesis inhibitors (unpublished).

In the present experiment, body weight gain was decreased and kidney weight was increased by E.P. administration in all cases. Sullivan and Smith [36] and Meites [37] have already reported that the decrease of body weight gain by estrogen administration for a longer period (40–100 days) to young rats was induced by the decrease of food intake. It should be noted here that the decrease in body weight gain occurred within 7 days after the start of E.P. administration, even in adult rats. This may be the main difference from the above two reports. The mechanism by which the kidney weight was increased by E.P. has not been clearly demonstrated, because the effects of estrogens on kidney weight differ by animal species and sexual differences [38]. As the tubular reabsorption of urinary sodium is accelerated by E_2 [39], one of the reasons for the increases in kidney weight by E.P. administration may be due to the retention of sodium and water in the kidney itself, because the kidneys of E.P.-treated rats swelled and were soft in consistency at autopsy compared with those of controls.

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