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EXPERIMENTAL STUDIES ON THE CARBONIC ANHYDRASE ACTIVITY—XI.

EFFECT OF ADRENOCORTICOSTEROIDS ON CARBONIC ANHYDRASE AND Na^+–K^+–ACTIVATED ADENOSINE TRIPHOSPHATASE FROM KIDNEY SUBCELLULAR FRACTIONS IN NORMAL MICE AND RATS

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Abstract—The effects of adrenocorticosteroids on renal carbonic anhydrase and Na^+–K^+–ATPase activities in normal mice and rats were examined. Carbonic anhydrase activity was increased, while Na^+–K^+–ATPase activity was decreased after injection of aldosterone in mice. On the other hand, carbonic anhydrase activity was decreased, while Na^+–K^+–ATPase activity was increased with aldosterone in rats. Spironolactone blocked the action of aldosterone on carbonic anhydrase and Na^+–K^+–ATPase activities in mice, but in rats its blocking effect on Na^+–K^+–ATPase activity was not observed. DOCA increased carbonic anhydrase activity without any effect on Na^+–K^+–ATPase activity in both animal species.

Corticosterone and cortisol did not affect carbonic anhydrase activity in either animal species. Corticosterone increased Na^+–K^+–ATPase activity in mice, but decreased its enzymic activity in rats. While cortisol decreased Na^+–K^+–ATPase activity in mice, but increased its enzymic activity in rats. Species differences were observed in the action of adrenocorticosteroids on renal carbonic anhydrase and Na^+–K^+–ATPase activities in mice and rats.

ADRENOCORTICOSTEROIDS play an important role in the regulation of the sodium and potassium balance. Aldosterone acts on the kidney and decreases sodium excretion. Cortisol also induces sodium retention and an increase in potassium excretion, but much less effectively than aldosterone.

The mechanism of action of adrenocorticosteroids on renal enzyme system has not been clearly established. An increase in the activities of succinate dehydrogenase and cytochrome oxidase in rat kidney after injection of aldosterone *in vivo* has been reported,[1] but similar results could not be observed *in vitro*.[2] The Na^+–K^+–ATPase activity in rat kidney microsome is diminished after adrenalectomy and adrenocorticosteroids prevent the decline in its enzymic activity associated with adrenalectomy.[3–5]

Carbonic anhydrase is highly concentrated in the cortex of kidney[6] and this enzyme system has been shown to play a role in the formation of H^+ and on K^+–Na^+ or H^+–Na^+ exchange mechanism in renal tubules.[7, 8] Recently Na^+–K^+–ATPase is considered to be an important enzyme in Na^+ transport in the kidney.[9]

In view of these observations we considered it of interest to study the interaction of adrenocorticosteroids, especially aldosterone, with carbonic anhydrase and Na^+–K^+-ATPase of the kidney.

MATERIALS AND METHODS

Animals

Adult male ddN strain mice weighing about 30 g and male Wistar strain rats weighing about 250 g were fed solid diet (Oriental Co.) and tap water *ad libitum* and housed in a room at about 20°.

Drug administration

DL-aldosterone-21-monoacetate (Ciba) and deoxycorticosterone acetate (DOCA; Takeda) were dissolved in sesame oil. Corticosterone (N.B. Co.) and cortisol acetate (Merck) were suspended in Aqueous Vehicle No. 1 (Merck). Various doses of these hormones were administered S.C. in 0·1 ml of each solvent once daily for 4 days into the back of animals. Spironolactone (Aldacton A; Dainippon Seiyaku Co.) was suspended in distilled water (0·5 mg/0·1 ml) and administered orally once or twice daily for 4 days.

Preparation of subcellular fractions

In this experiment, whole kidney in mice and kidney cortex in rats were used. After sacrifice by decapitation, kidneys were removed and minute incisions were made and washed well with cold distilled water which was then absorbed with blotting paper. After repeating this procedure several times and removing containing blood as much as possible, the kidneys of animals in a group were pooled and homogenized in a Potter–Elvehjem homogenizer fitted with a Teflon pestle with 9 vol. of 0·25 M sucrose (solution A) or 0·25 M sucrose containing 0·1% sodium deoxycholate and 5 mM Na_2-EDTA at pH 7·4 with 1 M Tris (solution B). Fractionation of kidney homogenate was made according to Schneider.[10] Twenty ml of homogenate was centrifuged with a refrigerated centrifuge at 0° for 10 min (700 *g*) to sediment the nuclei and unbroken cells. The sediment was resuspended in 8·0 ml of solution A or B, and recentrifuged as above. The final sediment was resuspended in 0·25 M sucrose and labeled the nuclear fraction. The mixture of supernatant and washing of nuclear fraction was centrifuged at 0° for 10 min (9000 *g*) to sediment the mitochondria. The sediment was resuspended in 8·0 ml of solution A or B, and recentrifuged as above. The final sediment was resuspended in 0·25 M sucrose and labeled the mitochondrial fraction. In many cases, homogenate was centrifuged immediately for 10 min (9000 *g*) to sediment the nuclei together with mitochondria. The sediment was resuspended in 8·0 ml of solution A or B and recentrifuged as above. The mixture of supernatant and washing of mitochondria was centrifuged at 0° for 1 hr (77,000 *g*) to sediment the submicroscopic particles. The sediment was resuspended in 0·25 M sucrose and labeled the microsomal fraction. The supernatant obtained from 77,000 *g* centrifugation was used as the supernatant fraction.

Carbonic anhydrase assay

Carbonic anhydrase activity was measured according to Altschule and Levis.[11] The procedure was as follows; 1·0 ml of 0·2 M phosphate buffer (pH 6·8) and 0·5 ml of

enzyme preparation was placed in one compartment of boat-shaped glass vessel similar to Meldrum and Roughton[12] and in the other compartment 1·0 ml of 0·05 M $NaHCO_3$ solution was added. The reaction vessel containing the reagents and enzyme was attached to its manometer and placed in a water bath for 8 min at 37°, then the vessel was shaken at 120 cpm and the CO_2 produced was determined manometrically. Assays were made in several times with same enzyme preparation and mean values were calculated.

Na^+– and K^+– activated, Mg^{2+}– dependent adenosine triphosphatase (Na^+–K^+–ATPase) assay

Na^+–K^+–ATPase activity was estimated in tubes (100 × 15 mm) containing 1·8 ml medium and 0·2 ml enzyme preparation, the total volume of reaction mixture was kept constant at 2·0 ml. The reaction mixture always contained constituents below other than enzyme preparation, 25 mM Tris-HCl buffer (pH 7·4); 3 mM ATP, rendered sodium free by treatment with Dowex 50 resin (H^+ form) according to Schwartz *et al.*[13] and brought to pH 7·4 with 1 M Tris; 100 mM NaCl; 20 mM KCl and 5 mM $MgCl_2$. They were shaken at 37° for several minutes at 120 cpm in incubator, then the tube was placed in ice and the reaction was stopped by the addition of 1·0 ml of 30% trichloroacetic acid, and liberated inorganic phosphate (Pi) was determined by the method of Allen,[14] with the slight modification described by Nakamura.[15] Assays were made in several times with same enzyme preparation and mean values were calculated. Enzymic activity was expressed as μmole of Pi liberated per 1 mg protein.

Protein assay

Protein amount of enzyme preparation was determined by Biuret reaction[16] with crystalline bovine serum albumin (Sigma Chem. Co.) used as protein standard.

RESULTS

Experiments with mice

Effect of aldosterone on carbonic anhydrase. In Table 1 is presented the effect of aldosterone on the carbonic anhydrase activity in mice. The administration of 2 μg of aldosterone per mouse per day for 4 days resulted in a significant increase in carbonic anhydrase activity in homogenate, microsomal and supernatant fractions.

TABLE 1. EFFECT OF ALDOSTERONE ON CARBONIC ANHYDRASE FROM MOUSE KIDNEY

Group	No. of animals	Carbonic anhydrase activity: mean ± S.D. Enzyme unit/mg protein		
		Homogenate	Microsome	Supernatant
Control	7	0·29 ± 0·02 (6)	0·28 ± 0·03 (6)	1·05 ± 0·03 (5)
Aldosterone 2 μg × 4	7	0·37 ± 0·02* (6)	0·36 ± 0·01* (6)	1·47 ± 0·05* (5)

Numbers in parentheses represent the number of observations. Kidneys were homogenized with 0·25 M sucrose containing 0·1% sodium deoxycholate and 5 mM Na_2-EDTA.

* $P < 0·01$.

Effect of aldosterone on microsomal Na^+–K^+–ATPase. Figs. 1(a) and 1(b) show the Na^+–K^+–ATPase activity at various incubation times in control and aldosterone treated groups. In preparation for enzyme sample, the kidneys of 20 animals were provided in both groups respectively. In Fig. 1(a) 1 μg and in Fig. 1(b) 2 μg of aldosterone was administered per mouse per day for 4 days. The administration of 1 μg and 2 μg of aldosterone resulted in a significant decrease in Na^+–K^+–ATPase activity ($P < 0.05$) at any incubation time compared with the control.

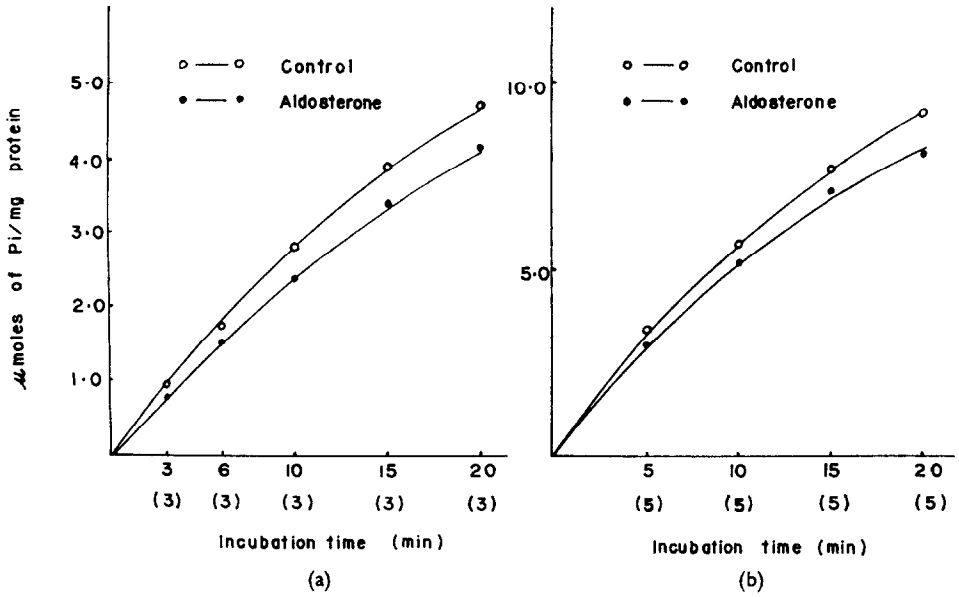

FIG. 1. The effect of aldosterone on microsomal Na^+–K^+–ATPase activity from mouse kidney. Numbers in parentheses represent the number of observations. (a) Kidneys were homogenized with 0·25 M sucrose. (b) Kidneys were homogenized with 0·25 M sucrose containing 0·1% sodium deoxycholate and 5 mM Na_2–EDTA.

Effect of spironolactone on carbonic anhydrase and Na^+–K^+–ATPase. The effect of spironolactone on carbonic anhydrase and Na^+–K^+–ATPase is summarized in Table 2. The administration of spironolactone (0·5 mg/mouse/day for 4 days) resulted in an increase in Na^+–K^+–ATPase activity in both cases (in one case kidneys were homogenized with 0·25 M sucrose and in another case kidneys were homogenized with 0·25 M sucrose containing 0·1% sodium deoxycholate and 5 mM Na_2-EDTA). On the other hand, carbonic anhydrase activity was decreased in every fraction. These results were reverse to the results indicated in Table 1 and Fig. 1.

Effect of DOCA on carbonic anhydrase and Na^+–K^+–ATPase. In Table 3 is presented the effect of DOCA treatment (0·1 mg or 0·5 mg/mouse/day for 4 days) upon the activities of both enzymes. Carbonic anhydrase activity was increased by a dose of 0·1 mg and 0·5 mg of DOCA in every fraction, while Na^+–K^+–ATPase activity was not affected in both experiments.

Effect of corticosterone and cortisol on carbonic anhydrase and Na^+–K^+–ATPase. As shown in Table 4, the administration of 0·5 mg corticosterone per mouse per day for 4 days did not affect carbonic anhydrase in every fraction with an increase in Na^+–K^+–ATPase activity. Both 0·1 mg and 0·5 mg of cortisol/mouse/day for 4 days

did not affect carbonic anhydrase activity in every fraction. The administration of 0·1 mg cortisol decreased Na^+–K^+–ATPase activity, while 0·5 mg of cortisol did not affect its enzymic activity.

TABLE 2. EFFECT OF SPIRONOLACTONE ON CARBONIC ANHYDRASE AND Na^+–K^+–ATPASE FROM MOUSE KIDNEY

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein Homogenate	Microsome	Microsome ATPase activity* μmoles Pi/mg protein Supernatant	/20 min
Control†	6	0·31 ± 0·02 (5)	0·34 ± 0·02 (5)	1·26 ± 0·10 (5)	5·19 ± 0·56 (5)
Spironolactone† 0·5 mg × 4	6	0·25 ± 0·03§ (5)	0·23 ± 0·02‖ (5)	0·85 ± 0·12‖ (5)	6·78 ± 0·48‖ (5)
Control‡	6	0·32 ± 0·03 (5)	0·39 ± 0·02 (5)	0·97 ± 0·04 (5)	8·83 ± 0·35 (6)
Spironolactone‡ 0·5 mg × 4	6	0·27 ± 0·02‖ (5)	0·28 ± 0·04‖ (5)	0·54 ± 0·06‖ (5)	9·29 ± 0·20§ (6)

* Mean ± S.D. Numbers in parentheses represent the number of observations.

† Kidneys were homogenized with 0·25 M sucrose.

‡ Kidneys were homogenized with 0·25 M sucrose containing 0·1% sodium deoxycholate and 5 mM Na_2-EDTA.

§ $P < 0·05$.

‖ $P < 0·01$.

TABLE 3. EFFECT OF DOCA ON CARBONIC ANHYDRASE AND Na^+–K^+–ATPASE FROM MOUSE KIDNEY

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein Homogenate	Microsome	Microsome ATPase activity* μmoles Pi/mg protein Supernatant	/20 min
Control†	16	0·26 ± 0·03 (7)	0·31 ± 0·02 (5)	1·10 ± 0·02 (6)	5·15 ± 0·27 (6)
DOCA† 0·1 mg × 4	16	0·42 ± 0·06§ (7)	0·40 ± 0·06§ (5)	1·61 ± 0·02§ (6)	5·19 ± 0·39 (6)
Control‡	7	0·29 ± 0·02 (6)	0·28 ± 0·03 (6)	1·05 ± 0·03 (5)	8·51 ± 0·49 (5)
DOCA‡ 0·5 mg × 4	7	0·36 ± 0·01§ (6)	0·38 ± 0·03§ (6)	1·34 ± 0·06§ (5)	8·21 ± 0·15 (5)

* Mean ± S.D. Numbers in parentheses represent the number of observations.

† Kidneys were homogenized with 0·25 M sucrose.

‡ Kidneys were homogenized with 0·25 M sucrose containing 0·1% sodium deoxycholate and 5 mM Na_2-EDTA.

§ $P < 0·01$.

Experiments with rats

Effect of aldosterone on carbonic anhydrase. In Table 5 is presented the effect of aldosterone on the carbonic anhydrase activity in rats. The administration of aldosterone (5 μg/rat/day for 4 days) decreased the enzymic activities in homogenate, nuclear, mitochondrial, microsomal and supernatant fractions. The administration of 10 μg aldosterone (per rat per day for 4 days) also decreased the enzymic activities in homogenate and microsomal fraction except in supernatant fraction, in which its enzymic activity was not changed.

Effect of aldosterone on microsomal Na^+–K^+–ATPase. Figs. 2(a) and 2(b) show the changes of Na^+–K^+–ATPase activity at various incubation times in control and aldosterone treated groups. In both experiments, the kidneys of 7 animals were provided for preparation of enzyme sample in control and experimental groups

TABLE 4. EFFECT OF CORTICOSTERONE AND CORTISOL ON CARBONIC ANHYDRASE AND Na^+–K^+–ATPASE FROM MOUSE KIDNEY

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein Homogenate	Microsome	Microsome ATPase activity* μmoles Pi/mg protein Supernatant	/20 min
Control‡	8	0·35 ± 0·02 (5)	0·43 ± 0·05 (5)	0·95 ± 0·09 (5)	10·50 ± 0·50 (6)
Corticosterone‡ 0·5 mg × 4	8	0·35 ± 0·01 (5)	0·42 ± 0·03 (5)	0·98 ± 0·04 (5)	11·54 ± 0·35‖ (6)
Control†	16	0·26 ± 0·03 (7)	0·31 ± 0·02 (5)	1·10 ± 0·02 (6)	5·15 ± 0·27 (6)
Cortisol† 0·1 mg × 4	16	0·26 ± 0·02 (6)	0·30 ± 0·02 (5)	1·18 ± 0·02 (7)	4·86 ± 0·09§ (6)
Control‡	7	0·29 ± 0·02 (6)	0·28 ± 0·03 (6)	1·05 ± 0·03 (5)	8·51 ± 0·49 (5)
Cortisol‡ 0·5 mg × 4	7	0·27 ± 0·02 (6)	0·27 ± 0·02 (6)	0·98 ± 0·05 (5)	8·67 ± 0·16 (5)

* Mean ± S.D. Numbers in parentheses represent the number of observations.

† Kidneys were homogenized with 0·25 M sucrose.

‡ Kidneys were homogenized with 0·25 M sucrose containing 0·1% dosium deoxycholate and 5 mM Na_2-EDTA.

§ $P < 0.05$.

‖ $P < 0.01$.

TABLE 5. EFFECT OF ALDOSTERONE ON CARBONIC ANHYDRASE FROM RAT KIDNEY

Group	No. of animals	Carbonic anhydrase activity: Mean ± S.D. Enzyme unit/mg protein Homogenate	Nuclei	Mitochondria	Microsome	Supernatant
Control*	7	0·41 ± 0·03 (5)	0·17 ± 0·03 (4)	0·18 ± 0·01 (4)	0·31 ± 0·01 (4)	0·92 ± 0·05 (4)
Aldosterone* 5 μg × 4	7	0·26 ± 0·06§ (4)	0·12 ± 0·01‡ (4)	0·14 ± 0·01§ (4)	0·20 ± 0·05§ (4)	0·75 ± 0·06§ (4)
Control†	5	0·43 ± 0·02 (6)			0·36 ± 0·02 (6)	1·09 ± 0·08 (6)
Aldosterone† 10 μg × 4	5	0·38 ± 0·02§ (6)			0·23 ± 0·02§ (6)	1·11 ± 0·09 (6)

Numbers in parentheses represent the number of observations.

* Kidneys were homogenized with 0·25 M sucrose.

† Kidneys were homogenized with 0·25 M sucrose containing 0·1% sodium deoxycholate and 5 mM Na_2-EDTA.

‡ $P < 0.05$.

§ $P < 0.01$.

respectively. In Fig. 2(a) 5 μg and in Fig. 2(b) 10 μg of aldosterone was administered per rat per day for 4 days. In Fig. 2(a) aldosterone increased Na^+–K^+–ATPase activity in any incubation time compared with the control. Fig. 2(b) shows the mean values of Na^+–K^+–ATPase activity in five observations. Ten μg of aldosterone significantly increased Na^+–K^+–ATPase activity ($P < 0.05$) in both incubation times.

Effect of spironolactone on carbonic anhydrase and Na⁺–K⁺–ATPase. As shown in Table 6, carbonic anhydrase activity was increased in homogenate, microsomal and supernatant fractions after the administration of spironolactone (5 mg per rat once

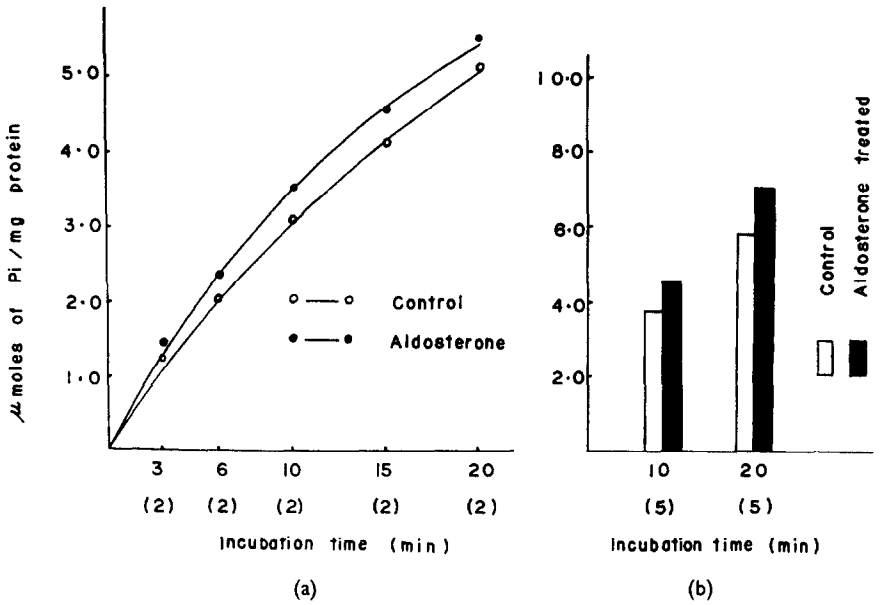

FIG. 2. The effect of aldosterone on microsomal Na^+–K^+–ATPase activity from rat kidney. Numbers in parentheses represent the number of observations. (a) Kidneys were homogenized with 0·25 M sucrose. (b) Kidneys were homogenized with 0·25 M sucrose containing 0·1 % sodium deoxycholate and 5 mM Na_2–EDTA

TABLE 6. EFFECT OF SPIRONOLACTONE ON CARBONIC ANHYDRASE AND Na^+–K^+–ATPASE FROM RAT KIDNEY

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein Homogenate	Microsome	Microsome ATPase activity* μmoles Pi/mg protein Supernatant	/20 min
Control	5	0·41 ± 0·04 (6)	0·43 ± 0·05 (6)	0·85 ± 0·02 (6)	11·75 ± 0·29 (6)
Spironolactone 5 mg × 4	5	0·55 ± 0·06‡ (6)	0·52 ± 0·03‡ (6)	1·08 ± 0·16‡ (6)	11·52 ± 0·23 (8)
Control	5	0·50 ± 0·03 (6)	0·74 ± 0·14 (6)	1·07 ± 0·14 (6)	12·60 ± 0·59 (6)
Spironolactone 1 mg × 8	5	0·72 ± 0·04‡ (6)	0·90 ± 0·07† (6)	1·57 ± 0·06‡ (6)	12·98 ± 0·53 (6)

* Mean ± S.D. Numbers in parentheses represent the number of observations.
Kidneys were homogenized with 0·25 M sucrose containing 0·1% sodium deoxycholate and 5 mM Na_2-EDTA.
† $P < 0·05$.
‡ $P < 0·01$.

daily for 4 days and 1 mg/rat twice daily for 4 days). On the other hand, Na^+–K^+–ATPase activity was not affected by spironolactone.

Effect of DOCA on carbonic anhydrase and Na⁺–K⁺–ATPase. The effect of the administration of DOCA on the carbonic anhydrase and Na^+–K^+–ATPase activities

is shown in Table 7. After the administration of DOCA (2 mg/rat/day for 4 days), carbonic anhydrase activity was increased in homogenate and microsomal fraction, but there was no increase in supernatant fraction. On the other hand, Na^+–K^+–ATPase activity was not affected after DOCA treatment.

TABLE 7. EFFECT OF DOCA ON CARBONIC ANHYDRASE AND Na^+–K^+–ATPASE FROM RAT KIDNEY

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein Homogenate	Microsome	Microsome ATPase activity* μmoles Pi/mg protein Supernatant	/20 min
Control	5	0·43 ± 0·02 (6)	0·36 ± 0·02 (6)	1·09 ± 0·08 (6)	8·03 ± 0·30 (5)
DOCA 2 mg × 4	5	0·51 ± 0·05† (5)	0·52 ± 0·05† (5)	1·06 ± 0·10 (5)	8·27 ± 0·30 (5)

* Mean ± S.D. Numbers in parentheses represent the number of observations.
Kidneys were homogenized with 0·25 M sucrose containing 0·1% sodium deoxycholate and 5 mM Na_2-EDTA.
† $P < 0·01$.

Effect of corticosterone and cortisol on carbonic anhydrase and Na^+–K^+–ATPase. As shown in Table 8, the administration of 2 mg corticosterone and cortisol (per rat per day for 4 days) did not affect carbonic anhydrase activity in every fraction. In corticosterone treated group Na^+–K^+–ATPase activity was decreased, while in cortisol treated group its enzymic activity was increased conversely.

TABLE 8. EFFECT OF CORTICOSTERONE AND CORTISOL ON CARBONIC ANHYDRASE AND Na^+–K^+–ATPASE FROM RAT KIDNEY

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein Homogenate	Microsome	Microsome ATPase activity* μmoles Pi/mg protein Supernatant	/20 min
Control	5	0·50 ± 0·02 (5)	0·71 ± 0·08 (5)	1·17 ± 0·10 (5)	14·09 ± 0·40 (5)
Corticosterone 2 mg × 4	5	0·50 ± 0·02 (5)	0·75 ± 0·08 (5)	1·15 ± 0·07 (5)	13·38 ± 0·47† (5)
Control	5	0·41 ± 0·04 (6)	0·43 ± 0·05 (6)	0·85 ± 0·02 (6)	11·75 ± 0·29 (6)
Cortisol 2 mg × 4	5	0·44 ± 0·04 (6)	0·47 ± 0·04 (6)	0·92 ± 0·15 (6)	12·57 ± 0·84† (8)

* Mean ± S.D. Numbers in parentheses represent the number of observations.
Kidneys were homogenized with 0·25 M sucrose containing 0·1% sodium deoxycholate and 5 mM Na_2-EDTA.
† $P < 0·05$.

DISCUSSION

Previously we observed that the effects of aldosterone on renal carbonic anhydrase activity were different in mice and rats being enhancing in the former and inhibitory in the latter.[17] To help explain above difference, the following hypothesis is offered: Presumably there is a species difference in the synthesis of renal carbonic anhydrase after aldosterone treatment and this difference may be dependent on the synthesis of other enzyme, for example Na^+–K^+–ATPase. In mice the increase in

carbonic anhydrase synthesis caused by aldosterone may cause a decrease in Na^+–K^+–ATPase synthesis and the situation may be reversed in rats. In the present experiment, the effects of adrenocorticosteroids, especially aldosterone, on renal carbonic anhydrase and Na^+–K^+–ATPase activities in normal mice and rats were investigated. The results agreed with our hypothesis.

DOCA increased carbonic anhydrase activity alone without any effect on Na^+–K^+–ATPase activity in both animal species. DOCA is considered to be similar to aldosterone in biological actions, but from the results in this experiment, the actions of DOCA and aldosterone on carbonic anhydrase and Na^+–K^+–ATPase seem to differ, but the reason for this is not clear.

Corticosterone and cortisol had no effects on carbonic anhydrase activity, but affected Na^+–K^+–ATPase activity in both animal species. This results seem to suggest the differences in the mechanism of action or in the site of action between aldosterone and corticosterone or cortisol.

Although the effect of aldosterone on sodium reabsorption by the kidney has been well documented, the mechanism of action or specific site of action of this hormone at the subcellular levels is not clear. The binding of ^{3}H-aldosterone to mitochondria was observed by Davidson *et al.*[18] Williams and Baba[19] using electrone microscope autoradiography reported that ^{3}H-aldosterone distributed in the proximal and distal convoluted tubules of rat kidney and bound mainly to mitochondria and plasma membranes. Also they supported the direct action of aldosterone on ATPase system or ATP production. On the other hand, Edelman and his associates[20–23] maintained that the aldosterone acts by increasing DNA-dependent RNA synthesis. Actinomycin D, puromycin and cycloheximide block the action of aldosterone in sodium transport correlating closely with the degree of inhibition of RNA and protein synthesis.[22] The renal nuclei contain the specific protein receptor for aldosterone in rat kidney.[23] Castles and Williamson[24] also stated that aldosterone enhances renal synthesis of RNA and actinomycin D blocks its synthesis and antinatriuretic action of the hormone in adrenalectomized rats. Recently the inhibiting action of actinomycin D on the uterine weight gain and elevation of carbonic anhydrase activity produced by oestrogen treatment of immature mice has been reported.[25]

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