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EXPERIMENTAL STUDIES ON THE CARBONIC ANHYDRASE ACTIVITY—XII

EFFECT OF ADRENOCORTICOSTEROIDS ON CARBONIC ANHYDRASE AND Na^+-K^+-ACTIVATED ADENOSINE TRIPHOSPHATASE FROM KIDNEY OF ADRENALECTOMIZED MICE AND RATS

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Abstract—The effects of adrenocorticosteroids administration on renal carbonic anhydrase and Na^+-K^+-ATPase activities in adrenalectomized animals were investigated.

In mice, carbonic anhydrase activities in homogenate, microsomal and supernatant fractions were decreased, while microsomal Na^+-K^+-ATPase activity was increased after adrenalectomy. In rats, the changes of both enzymic activities after adrenalectomy were reverse to those observed in mice. These changes in carbonic anhydrase activity after adrenalectomy were restored to normal levels with aldosterone and DOCA replacement, but corticosterone and cortisol had no effect on its enzymic activity in both animal species.

The elevation in Na^+-K^+-ATPase activity after adrenalectomy in mice was restored with DOCA and cortisol, but aldosterone had no effect on enzymic activity. The decline in Na^+-K^+-ATPase activity after adrenalectomy in rats was prevented and approached the normal levels with replacement of both mineralo- and glucocorticoids.

From above results it became clear that the adrenocorticosteroids have a role in maintaining normal levels of renal enzymic activities.

ALTHOUGH the action of adrenocorticosteroids, especially aldosterone, in animal kidney has been considered to regulate sodium and potassium transport in renal tubules, there has been no systematic investigation on the relationships between aldosterone and kidney enzymes.

Recently, the effects of aldosterone on the activities of succinate dehydrogenase and cytochrome oxidase in rat kidney were reported.[1–3] The interrelations between renal Na^+-K^+-ATPase activity and adrenocorticosteroids in adrenalectomized rats were also investigated.[4–6] Katz and Epstein[7] reported that there is a close correlation between microsomal Na^+-K^+-ATPase activity and sodium reabsorption in rat kidney and then adrenalectomy decrease this activity and reabsorption of sodium in renal tubules.

Previously, the authors examined the effects of adrenocorticosteroids on renal carbonic anhydrase and Na^+-K^+-ATPase activities in normal mice and rats.[8] The present experiment has been done to clarify the replacement effects of aldosterone and other adrenocorticosteroids on the changes of carbonic anhydrase and Na^+-K^+-ATPase activities in animals with adrenocortical insufficiency.

MATERIALS AND METHODS

Animals

Adult male, adrenalectomized ddN strain mice weighing about 25 g and adrenalectomized Wistar strain rats weighing about 220 g were used. The animals were fed with commercial solid diet (Oriental Co.) and tap water *ad libitum* at room temperature of about 24°. Bilateral adrenalectomy was carried out under ether anesthesia through a dorsal route.

Hormone administration

DL-aldosterone-21-monoacetate (Ciba) and deoxycorticosterone acetate (DOCA; Takeda Co.) 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 in 0·1 ml solvent were administered subcutaneously into the back. In many cases, the administration started from the next day after adrenalectomy and was repeated once daily for several days. The animals were sacrificed 24 hr after the last injection of hormones.

Separation of subcellular fractions

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 as much blood as possible, the kidneys of animals in a group were pooled, weighed with a torsion balance and homogenized in a Potter–Elvehjem type glass homogenizer fitted with a Teflon pestle with 9 vol. of 0·25 M sucrose containing 0·1 % sodium deoxycholate and 5 mM Na_2EDTA adjusted to pH 7·4 with 1 M Tris (solution A). Fractionation of kidney homogenate was made according to Schneider.[9]

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 supernatant was decanted and the sediment was resuspended in solution A and recentrifuged under the same conditions. The supernatant solutions from both centrifugations were then combined and centrifuged at 0° for 10 min (9000 *g*) to sediment the mitochondria. The sediment was resuspended in solution A and recentrifuged as above. In many cases, the homogenate was centrifuged immediately for 10 min (9000 *g*) to sediment the nuclei together with mitochondria. The supernatant was decanted and the sediment was washed once with solution A and recentrifuged as above. The supernatant solutions from both centrifugations were then combined and centrifuged at 0° for 1 hr (77,000 *g*) using ultracentrifuge to sediment the submicroscopic particles. The sediment was suspended in 0·25 M sucrose and called 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.[10] 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 a boat-shaped glass vessel and in the other compartment 1·0 ml of 0·05 M $NaHCO_3$ solution was added. The

reaction vessel containing the reagents and the enzyme was attached to its manometer and placed in a water bath for 8 min at 37°, then the vessel was shaken 120 times/min and the CO_2 produced was determined manometrically. Assays were made several times with the same enzyme preparation and mean values were calculated.

Na^+- and K^+-activated, Mg^{++}-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 the following constituents 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.*[11] 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 20 min 120 times/min in incubator, then the tubes were 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,[12] with the slight modification described by Nakamura.[13] Assays were made several times with the same enzyme preparation and mean values were calculated. Enzymic activity was expressed as micromoles of Pi liberated per milligram protein.

Protein assay

Protein amount of enzyme preparation was determined by Biuret reaction[14] with crystalline bovine serum albumin (Sigma Chem. Co.) used as protein standard.

RESULTS

Experiments with adrenalectomized mice

Effect of adrenalectomy. Figure 1 shows the time course of the changes of carbonic anhydrase and Na^+-K^+-ATPase activities in mouse kidney microsomes after

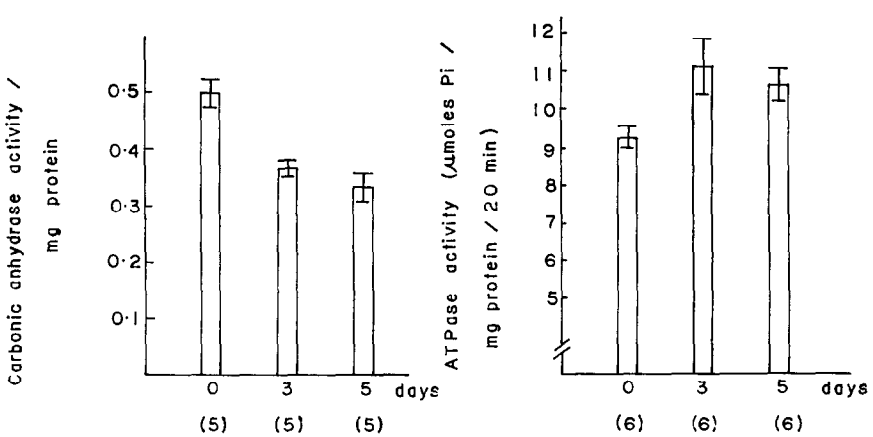

FIG. 1. The effect of adrenalectomy on microsomal carbonic anhydrase and Na^+-K^+-ATPase levels from mouse kidney. Seven mice in each group were operated on day zero and then sacrificed on the days indicated. Numbers in parentheses represent the number of observations. The mean value is given by the height of the bar with the standard deviation represented as vertical lines.

adrenalectomy. Carbonic anhydrase activity was decreased gradually and significantly ($P < 0.05$) after operation. On the other hand, Na^+-K^+-ATPase activity was increased on the third and fifth postoperative day ($P < 0.05$).

Effect of aldosterone administration. In order to investigate the replacement effect of aldosterone on the alterations of both enzymic activities produced by adrenalectomy, experiments were carried out under two conditions. (1) In this case, various doses of aldosterone were administered per mouse per day for 3 days from the next day after adrenalectomy. As shown in Table 1, carbonic anhydrase activity was increased gradually parallel with increase in a dose of aldosterone and returned to the normal levels in every fraction. On the other hand, aldosterone had no significant effect on Na^+-K^+-ATPase activity. (2) In this case, 1 μg of aldosterone was administered per mouse per day for 2 days from the third day after adrenalectomy. As shown in Fig. 2, carbonic anhydrase activities in homogenate, microsomal and supernatant fractions were all increased significantly ($P < 0.05$) and restored to the control levels in aldosterone treated group. On the other hand, an effect of aldosterone on microsomal Na^+-K^+-ATPase activity could not be observed.

Effect of DOCA administration. Table 2 shows the effect of DOCA administration (per mouse per day for 3 days from the next day after adrenalectomy) on carbonic anhydrase and Na^+-K^+-ATPase activities. Carbonic anhydrase activities in homo-

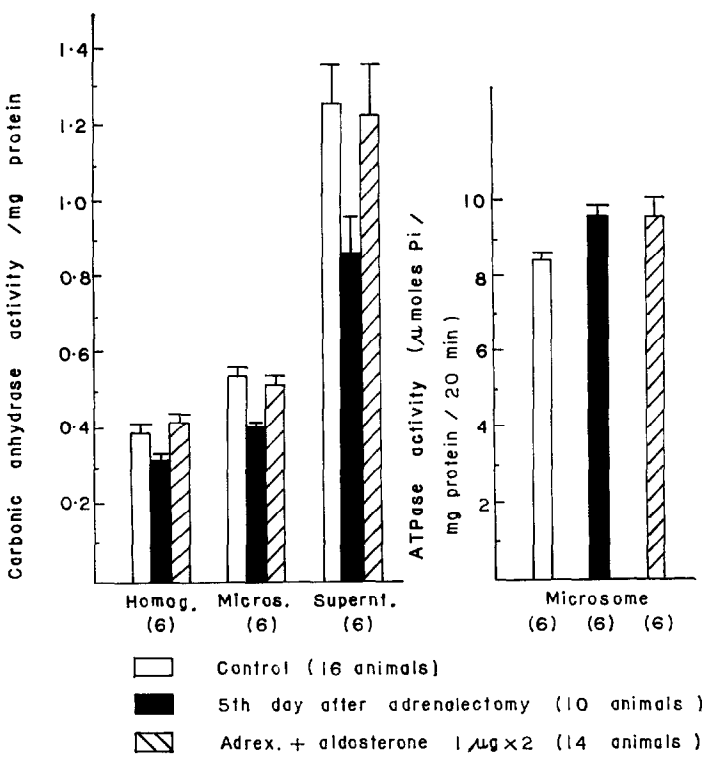

FIG. 2. The effect of aldosterone on kidney carbonic anhydrase and Na^+-K^+-ATPase levels in adrenalectomized mice. Numbers in parentheses represent the number of observations. The mean value is given by the height of the bar with the standard deviation represented as a vertical line.

TABLE 1. EFFECT OF ALDOSTERONE ADMINISTRATION ON CARBONIC ANHYDRASE AND Na^+-K^+-ATPASE IN ADRENALECTOMIZED MICE

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein			Microsome ATPase activity* (μmoles Pi/mg protein/20 min)
		Homogenate	Microsome	Supernatant	
Control	8	0·36 ± 0·01 (5)	0·56 ± 0·03 (5)	1·26 ± 0·01 (5)	7·57 ± 0·30 (5)
5th day after adrex.	7	0·25 ± 0·02† (5)	0·39 ± 0·05† (5)	0·69 ± 0·01† (5)	8·54 ± 0·26† (5)
Adrex. + ald. 0·08 μg × 3	7	0·34 ± 0·01‡ (5)	0·55 ± 0·05‡ (5)	0·94 ± 0·10‡ (5)	8·51 ± 0·31 (5)
Adrex + ald. 0·4 μg × 3	8	0·38 ± 0·04‡ (5)	0·62 ± 0·05‡ (5)	1·12 ± 0·10‡ (5)	8·43 ± 0·14 (5)
Adrex. + ald. 2 μg × 3	8	0·40 ± 0·01‡ (5)	0·67 ± 0·04‡ (5)	1·35 ± 0·07‡ (5)	8·34 ± 0·19 (5)

* Mean ± S.D. Numbers in parentheses represent the number of observations.
† $P < 0·05$ (when compared with control group).
‡ $P < 0·05$ (when compared with adrex. group).

TABLE 2. EFFECT OF DOCA ADMINISTRATION ON CARBONIC ANHYDRASE AND Na^+-K^+-ATPASE IN ADRENALECTOMIZED MICE

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein			Microsome ATPase activity* (μmoles Pi/mg protein/20 min)
		Homogenate	Microsome	Supernatant	
Control	9	0·38 ± 0·02 (5)	0·63 ± 0·03 (6)	0·96 ± 0·07 (6)	8·44 ± 0·44 (5)
5th day after adrex.	8	0·30 ± 0·02† (5)	0·47 ± 0·06† (6)	0·73 ± 0·05† (6)	9·74 ± 0·47† (5)
Adrex. + DOCA 20 μg × 3	8	0·38 ± 0·02‡ (5)	0·56 ± 0·05‡ (6)	0·95 ± 0·05‡ (6)	8·33 ± 0·28‡ (5)
Adrex. + DOCA 100 μg × 3	8	0·40 ± 0·04‡ (5)	0·56 ± 0·05‡ (6)	1·04 ± 0·08‡ (6)	9·10 ± 0·65 (5)
Adrex. + DOCA 500 μg × 3	9	0·40 ± 0·01‡ (5)	0·68 ± 0·05‡ (6)	1·13 ± 0·08‡ (6)	8·96 ± 0·28‡ (5)

* Mean ± S.D. Numbers in parentheses represent the number of observations.
† $P < 0·05$ (when compared with control group).
‡ $P < 0·05$ (when compared with adrex. group).

genate and supernatant fraction returned to the control levels with 20 μg DOCA. Larger dose of DOCA (500 μg $\times$ 3) increased enzymic activity in supernatant fraction over the control levels. Microsomal Na^+-K^+-ATPase activity, elevated after adrenalectomy, was diminished to the control levels with 20 μg of DOCA, but the decrease in enzymic activity was less with 100 and 500 μg of DOCA.

Effect of cortisol administration. In Table 3 is presented the effect of cortisol administration (per mouse per day for 3 days from the next day after adrenalectomy). Various doses of this hormone had no effect on carbonic anhydrase activities in any fraction. Na^+-K^+-ATPase activity, elevated after adrenalectomy, was decreased to the control levels with 20 μg of cortisol, but enzymic activity did not change after the administration of 100 and 500 μg cortisol.

Experiments with adrenalectomized rats

Effect of adrenalectomy. As shown in Fig. 3, carbonic anhydrase activities in homogenate, microsomal and supernatant fractions were all increased significantly ($P < 0{\cdot}05$) after adrenalectomy. On the sixth postoperative day, changes in enzymic activity were similar to those observed on the fourth postoperative day. Figure 3 shows also the changes of microsomal Na^+-K^+-ATPase activity after adrenalectomy. Enzymic activity was decreased gradually and significantly ($P < 0{\cdot}05$) according to the lapse of days after adrenalectomy.

Effect of aldosterone administration. In order to investigate the replacement effect of aldosterone on the changes of both enzymic activities produced by adrenalectomy, various amounts of aldosterone were administered per rat per day for 4 days from the next day after operation. As shown in Table 4, daily dose of 0·2 μg aldosterone

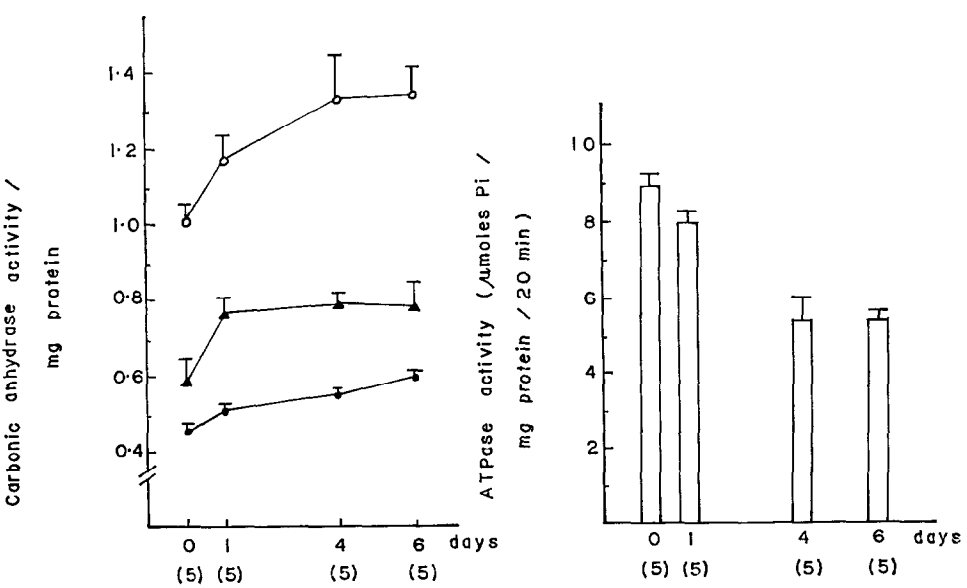

FIG. 3. The effect of adrenalectomy on carbonic anhydrase and Na^+-K^+-ATPase levels from rat kidney. Five rats in each group were operated on day zero and then sacrificed on the days indicated. Numbers in parentheses represent the number of observations. The mean value is given by the height of the point or bar with the standard deviation represented as a vertical line.
●—● Homogenate; ▲—▲ Microsome; ○—○ Supernatant.

TABLE 3. EFFECT OF CORTISOL ADMINISTRATION ON CARBONIC ANHYDRASE AND Na^+-K^+-ATPASE IN ADRENALECTOMIZED MICE

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein			Microsome ATPase activity* (μmoles Pi/mg protein/20 min)
		Homogenate	Microsome	Supernatant	
Control	8	0·43 ± 0·03 (5)	0·50 ± 0·02 (5)	1·43 ± 0·07 (6)	8·96 ± 0·45 (5)
5th day after adrex.	5	0·35 ± 0·02† (5)	0·42 ± 0·06† (5)	1·02 ± 0·08† (6)	10·48 ± 0·59† (5)
Adrex. + cortisol 20 μg × 3	7	0·36 ± 0·01 (5)	0·42 ± 0·04 (5)	0·98 ± 0·08 (6)	8·58 ± 0·18‡ (5)
Adrex. + cortisol 100 μg × 3	7	0·34 ± 0·02 (5)	0·45 ± 0·04 (5)	1·09 ± 0·02 (6)	9·96 ± 0·28 (5)
Adrex + cortisol 500 μg × 3	6	0·34 ± 0·02 (5)	0·39 ± 0·02 (5)	1·06 ± 0·05 (5)	9·88 ± 0·09 (5)

* Mean + S.D. Numbers in parentheses represent the number of observations.
† $P < 0·05$ (when compared with control group).
‡ $P < 0·05$ (when compared with adrex. group).

TABLE 4. EFFECT OF ALDOSTERONE ADMINISTRATION ON CARBONIC ANHYDRASE AND Na^+-K^+-ATPASE IN ADRENALECTOMIZED RATS

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein			Microsome ATPase activity* (μmoles Pi/mg protein/20 min)
		Homogenate	Microsome	Supernatant	
Control	5	0·53 ± 0·03 (5)	0·71 ± 0·03 (5)	1·15 ± 0·02 (5)	9·77 ± 0·18 (6)
6th day after adrex.	5	0·61 ± 0·04† (5)	0·95 ± 0·06† (5)	1·35 ± 0·08† (5)	7·62 ± 0·20† (6)
Adrex. + ald. 0·2 μg × 4	5	0·45 ± 0·03‡ (5)	0·91 ± 0·06 (5)	1·15 ± 0·07‡ (5)	7·44 ± 0·11 (6)
Adrex. + ald. 1·0 μg × 4	5	0·43 ± 0·04‡ (5)	0·71 ± 0·07‡ (5)	1·09 ± 0·10‡ (5)	8·33 ± 0·04‡ (6)
Adrex. + ald. 5·0 μg × 4	5	0·41 ± 0·02‡ (5)	0·54 ± 0·04‡ (5)	0·85 ± 0·07‡ (5)	8·52 ± 0·23‡ (6)

*Mean ± S.D. Numbers in parentheses represent the number of observations.
† $P < 0·05$ (when compared with control group).
‡ $P < 0·05$ (when compared with adrex. group).

TABLE 5. EFFECT OF DOCA ADMINISTRATION ON CARBONIC ANHYDRASE AND Na^+-K^+-ATPASE IN ADRENALECTOMIZED RATS

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein			Microsome ATPase activity* (μmoles Pi/mg protein/20 min)
		Homogenate	Microsome	Supernatant	
Control	6	0·48 ± 0·04 (5)	0·47 ± 0·08 (6)	1·10 ± 0·11 (6)	11·33 ± 0·16 (6)
6th day after adrex.	5	0·56 ± 0·03† (5)	0·87 ± 0·10† (5)	1·38 ± 0·13† (5)	8·68 ± 0·47† (6)
Adrex. + DOCA 1·5 mg × 4	5	0·49 ± 0·02‡ (5)	0·63 ± 0·07‡ (5)	1·08 ± 0·14‡ (5)	10·60 ± 0·48‡ (6)

* Mean ± S.D. Numbers in parentheses represent the number of observations.
† $P < 0.05$ (when compared with control group).
‡ $P < 0.05$ (when compared with adrex. group).

TABLE 6. EFFECT OF CORTICOSTERONE ADMINISTRATION ON CARBONIC ANHYDRASE AND Na^+-K^+-ATPASE IN ADRENALECTOMIZED RATS

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein			Microsome ATPase activity* (μmoles Pi/mg protein/20 min)
		Homogenate	Microsome	Supernatant	
Control	5	0·53 ± 0·03 (5)	0·71 ± 0·03 (5)	1·15 ± 0·02 (5)	9·77 ± 0·18 (6)
6th day after adrex.	5	0·61 ± 0·04† (5)	0·95 ± 0·06† (5)	1·35 ± 0·07† (5)	7·62 ± 0·20† (6)
Adrex. + corticosterone 0·4 mg × 4	5	0·61 ± 0·03 (5)	0·92 ± 0·05 (5)	1·30 ± 0·05 (5)	7·16 ± 0·23 (6)
Control	5	0·44 ± 0·02 (6)	0·46 ± 0·05 (6)	1·06 ± 0·04 (6)	8·55 ± 0·22 (6)
6th day after adrex.	5	0·52 ± 0·04† (6)	0·71 ± 0·04† (6)	1·35 ± 0·05† (6)	5·42 ± 0·85† (6)
Adrex. + corticosterone 2 mg × 4	5	0·47 ± 0·04 (6)	0·66 ± 0·10 (6)	1·40 ± 0·06 (6)	7·85 ± 0·37‡ (6)

* Mean ± S.D. Numbers in parentheses represent the number of observations.
† $P < 0.05$ (when compared with control group).
‡ $P < 0.05$ (when compared with adrex. group).

decreased carbonic anhydrase activities in homogenate and supernatant fraction without any significant effect in microsomal fraction, in which however, the enzymic activity restored to normal by a daily dose of 1·0 μg aldosterone. Daily dose of 5·0 μg aldosterone decreased carbonic anhydrase activities in all fractions below the control levels, and the decrease in microsomal and supernatant enzymic activities was almost in proportion to the injected dose of aldosterone. Na^+-K^+-ATPase activity declined after adrenalectomy and was increased towards the control levels with larger dose of aldosterone.

Effect of DOCA administration. In Table 5 is presented the effect of DOCA administration (1·5 mg/rat/day for 4 days from the next day after adrenalectomy) Carbonic anhydrase activities in homogenate, microsomal and supernatant fractions were all decreased with DOCA. On the other hand, Na^+-K^+-ATPase activity was increased and approached the control levels with DOCA treatment.

Effect of corticosterone administration. As shown in Table 6, both 0·4 and 2·0 mg of corticosterone/rat/day for 4 days from the next day after adrenalectomy had no effect on carbonic anhydrase activities in homogenate, microsomal and supernatant fractions. Larger dose of corticosterone increased Na^+-K^+-ATPase activity to near the control levels.

Effect of cortisol administration. In Table 7 is presented the effect of cortisol administration (2·0 mg/rat/day for 4 days from the next day after adrenalectomy). Cortisol had no effect on carbonic anhydrase activities in any fraction similarly to the previous experiment. On the other hand, Na^+-K^+-ATPase activity was increased significantly and approached the control levels after cortisol treatment.

DISCUSSION

In this experiment, we have examined the carbonic anhydrase and Na^+-K^+-ATPase, which are thought to be related to sodium and potassium metabolism in the kidney.[15–17] The changes of both enzymic activities following adrenalectomy were opposite in mice and rats.

It was reported that the renal carbonic anhydrase activity was elevated following adrenalectomy and treatment with aldosterone decreased enzymic activity to normal levels in the rabbit, but this enzymic activity did not change after adrenalectomy in mouse.[18] Arima[19] reported that the renal carbonic anhydrase activity did not change in rats adrenalectomized or treated with DOCA. These results did not agree with our results. According to recent observations,[4–6] Na^+-K^+-ATPase activity in rat kidney microsomes was diminished after adrenalectomy and adrenocorticosteroids administration prevented the decline and restored enzymic activity to the normal levels. These results agreed with our findings in this experiment.

We have recently observed with normal mice and rats that the renal carbonic anhydrase activity was affected by aldosterone and DOCA, but was not affected by corticosterone and cortisol. On the other hand, microsomal Na^+-K^+-ATPase activity was affected by aldosterone, corticosterone and cortisol, but DOCA had no effect on this enzymic activity.[8] The data provided here indicate that, in adrenalectomized mice and rats, aldosterone and DOCA maintain normal renal carbonic anhydrase levels following adrenalectomy. On Na^+-K^+-ATPase activity, aldosterone and DOCA showed a replacement effect in rats, but a similar effect could not be observed with aldosterone in mice. The physiological significance of this difference is not clear at

TABLE 7. EFFECT OF CORTISOL ADMINISTRATION ON CARBONIC ANHYDRASE AND Na^+-K^+-ATPASE IN ADRENALECTOMIZED RATS

Group	No. of animals	Carbonic anhydrase activity* Enzyme unit/mg protein			Microsome ATPase activity* (μmoles Pi/mg protein/20 min)
		Homogenate	Microsome	Supernatant	
Control	5	0·41 ± 0·04 (6)	0·43 ± 0·05 (6)	1·12 ± 0·02 (6)	11·87 ± 0·20 (6)
6th day after adrex.	5	0·60 ± 0·06† (6)	0·54 ± 0·02† (6)	1·40 ± 0·09† (6)	8·68 ± 0·28† (6)
Adrex. + cortisol 2 mg × 4	5	0·58 ± 0·05 (6)	0·54 ± 0·04 (6)	1·44 ± 0·08 (6)	10·30 ± 0·48‡ (6)

* Mean ± S.D. Numbers in parentheses represent the number of observations.
† $P < 0·05$ (when compared with control group).
‡ $P < 0·05$ (when compared with adrex. group).

present. Presumably, the sensitivity of Na^+-K^+-ATPase to mineralocorticoids may be altered after adrenalectomy.

Recently, Edelman and his co-workers have maintained the following new theories concerning the mechanism of action of aldosterone. Aldosterone stimulates Na^+ transport by activating nuclear synthesis of DNA-dependent RNA and protein in toad bladder,[20] and in rats.[21] Aldosterone acts by inducing *de novo* synthesis of enzymes related to Na^+ transport.[22] The renal nuclei contain the specific protein receptors for aldosterone in rat kidney[23] and actinomycin D, puromycin and cycloheximide block the action of aldosterone on sodium transport correlating with the degree of inhibition of RNA and protein synthesis.[24] Their experimental results may be related to our findings in this experiment.

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