Biochemical Pharmacology, Vol. 20, pp. 2191–2204. Pergamon Press, 1971. Printed in Great Britain

EXPERIMENTAL STUDIES ON THE CARBONIC ANHYDRASE ACTIVITY—XV

EARLY EFFECT OF ALDOSTERONE AND ACTINOMYCIN D ON CARBONIC ANHYDRASE AND ADENOSINE TRIPHOSPHATASE IN NORMAL AND ADRENALECTOMIZED RATS

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(*Received* 9 *September* 1970; *accepted* 2 *February* 1971)

Abstract—Effect of the single administration of aldosterone and actinomycin D on the activities of carbonic anhydrase in kidney and liver and of ATPase in kidney microsomes was examined with normal and adrenalectomized rats.

In normal rats, both aldosterone and actinomycin D alone inhibited kidney carbonic anhydrase activity and a minimum value was obtained in 4–6 hr after treatment. (Na^+–K^+–Mg^{2+})- and Mg^{2+}-ATPase activities were not affected by either drug. Aldosterone and a smaller dose of actinomycin D had a stimulatory effect, while a larger dose of actinomycin D showed an inhibitory effect on liver carbonic anhydrase activity.

Adrenalectomy produced an elevation in carbonic anhydrase activity of the kidney and liver and a decrease in (Na^+–K^+–Mg^{2+})-ATPase activity. Administration of aldosterone depressed the elevated carbonic anhydrase activity to normal level without any effect on ATPase activity. Although actinomycin D had no effect on carbonic anhydrase activity from kidney and liver of adrenalectomized rats; in aldosterone-pretreated adrenalectomized rats, actinomycin D inhibited carbonic anhydrase activity as in normal rats. Actinomycin D had an inhibitory effect on (Na^+–K^+–Mg^{2+})-ATPase activity in adrenalectomized and aldosterone-nontreated rats.

OF SEVERAL enzymes in the kidney, carbonic anhydrase is highly concentrated in the cortex of kidney[1] and this enzyme system has been shown to play a role in the formation of H^+ and on H^+–Na^+ or K^+–Na^+ exchange mechanism in renal tubules.[2,3] (Na^+– K^+)-activated adenosine triphosphatase also has been considered to be an important enzyme[4–6] in Na^+ transport in the kidney. On the other hand, the inhibitory effect of aldosterone on urinary excretion of sodium has been well documented and summarized by Tait and Tait.[7] However, there has been no systematic investigation on the relationships between aldosterone and these two kidney enzymes, especially carbonic anhydrase.

Previously,[8,9] we examined the alterations in the activities of above enzymes after repeated administration of aldosterone into normal and adrenalectomized rats and observed that aldosterone inhibited renal carbonic anhydrase activity, while this hormone had a stimulatory effect on (Na^+–K^+)-ATPase activity in the kidney microsomal fraction.

Recently, it has been reported that the maximal inhibitory effect of the single administration of aldosterone on urinary excretion of sodium in adrenalectomized rats appears in 2–4 hr after hormone treatment[10–12] and the inhibition of RNA

synthesis by actinomycin D abolishes the aldosterone-mediated increase of sodium transport in toad bladder[13] and rat kidney.[11,12] If carbonic anhydrase and ATPase are the mediators of the action of aldosterone in the kidney, some alterations in these enzymic activities may be observed during a period of maximal retention of sodium and the effect of aldosterone on these enzymes may be blocked by actinomycin D.

The present experiment was undertaken as an attempt to obtain more information on the mode of action of aldosterone and actinomycin D on both enzyme systems.

MATERIALS AND METHODS

Animals

Adult male Wistar strain rats, normal and adrenalectomized, weighing about 250 g were used. Bilateral adrenalectomy was carried out under ether anesthesia through dorsal route. The animals were fed with commercial solid diet (Oriental Co.) and tap water *ad lib.* at room temperature of 20° with normal rats and 24° with adrenalectomized rats. They were fasted for 24 hr before sacrifice being allowed only to drink water.

Drug administration

D-aldosterone (Mann) was dissolved in 95% ethanol and diluted with saline to adequate concentrations. Actinomycin D (Merck, Sharp & Dohme) was dissolved in saline. Various doses of these drugs were administered s.c. or i.p. in 0·1 ml/100 g body weight.

Separation of subcellular fractions

After sacrifice by decapitation, the liver and the cortex from kidney were removed, minute incisions were made and washed well with cold distilled water. After removing as much blood as possible, the liver and kidney cortex were homogenized in a Potter–Elvehjem type glass-teflon homogenizer 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. Differential fractionation was made according to Schneider[14] and 105,000 *g* precipitate (microsomal fraction) and 105,000 *g* supernatant (supernatant fraction) were obtained.

Carbonic anhydrase and ATPase assay

Carbonic anhydrase activity was manometrically measured according to Altschule and Levis[15] and the unit of enzymic activity was calculated by the method of Mitchell *et al.*[16] (Na^+–K^+–Mg^{2+})-activated adenosine triphosphatase (total-ATPase) activity was measured as described in the previous report.[8] In the assay of Mg^{2+}-activated adenosine triphosphatase (Mg^{2+}-ATPase) activity, NaCl and KCl were deleted from incubation medium and distilled water was added instead. The liberated inorganic phosphate (P_i) was determined by the method of Allen,[17] with the slight modification described by Nakamura[18] and the enzymic activity was expressed as micromoles of P_i liberated per miligram protein per 20 min. The amount of protein in the enzyme preparation was determined by Biuret reaction[19] with crystalline bovine serum albumin (Sigma Chem. Co.) used as protein standard.

RESULTS

Experiment with normal rats

Effect of aldosterone (Time course experiment). The animals were injected i.p. with 2 μg/kg of aldosterone and sacrificed at various times indicated in Figs. 1 and 2. As shown in the left half of Fig. 1, carbonic anhydrase activities in kidney microsomal

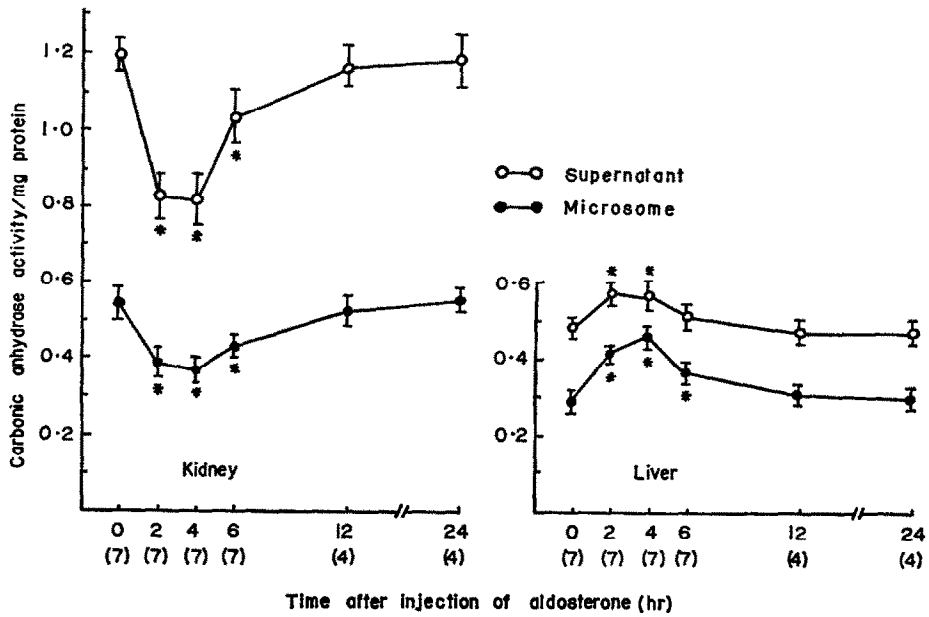

FIG. 1. Time course of response of carbonic anhydrase activity of kidney and liver to aldosterone. Each point with vertical lines represents the mean with standard deviation. Numbers in parentheses represent the number of animals. * $P < 0.05$ (when compared with 0 hr values).

and supernatant fractions were inhibited after aldosterone treatment and a maximal decrease was observed 4 hr later with a gradual return to normal levels by 12 hr. The enzymic activity in liver microsomal and supernatant fractions were elevated indicating a maximum within 2–4 hr after aldosterone and a return to normal level was seen at 12 hr later.

Figure 2 shows the changes of total- and Mg^{2+}-ATPase activities from kidney microsomes after aldosterone treatment. ATPase activities were not affected by aldosterone in any times.

Effect of actinomycin D (Time course experiment). Figure 3 shows the changes of carbonic anhydrase activity in kidney, liver and blood at intervals from 3 to 24 hr after single i.p. injection of 500 μg/kg of actinomycin D. In each group, five animals were used respectively. Carbonic anhydrase activities in kidney microsomal and supernatant fractions (left half of Fig. 3) were gradually decreased after the treatment of actinomycin D and a maximal depression was observed 6 hr later. Then, the enzymic activity was elevated and returned to normal levels by 24 hr. In the liver, carbonic anhydrase activities in both fractions were unaffected by this dose at any time after injection. Blood enzymic activity was decreased up to the 6th hr after actinomycin D

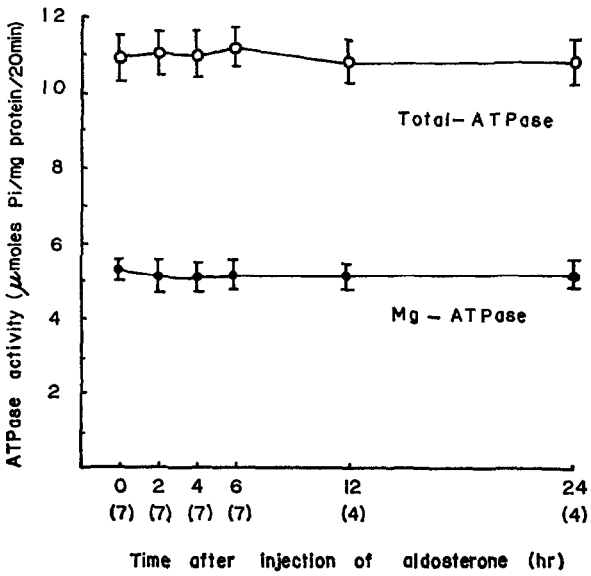

FIG. 2. Time course of response of kidney microsomal ATPase activity to aldosterone. Each point with vertical lines represents the mean with standard deviation. Numbers in parentheses represent the number of animals.

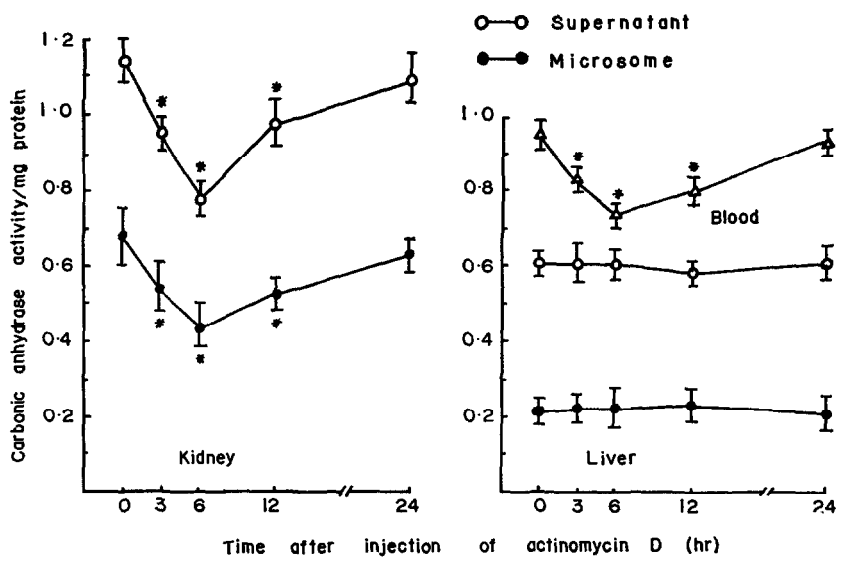

FIG. 3. Time course of response of carbonic anhydrase activity of kidney and liver to actinomycin D. Each point with vertical lines represents the mean with standard deviation. Blood enzymic activity (△—△) was measured with 0·5 ml of 100-fold diluted blood. * $P < 0.05$ (when compared with 0 hr values).

treatment and then returned to the normal level by 24 hr. On the other hand, actinomycin D had no significant effect on total-ATPase activity from kidney microsomes at any time after administration.

Effect of actinomycin D (Dose–response relation). In the previous report with mice,[20] we observed that the changes of liver carbonic anhydrase activity after actinomycin D treatment *in vivo* were different according to the administered doses, being stimulated by a smaller dose and inhibited by a larger dose. Therefore, the dose–response relation between actinomycin D and enzymic activity was examined.

Five animals in each group respectively were injected i.p. with actinomycin D in doses ranging from 62·5 to 1000 μg/kg and sacrificed 6 hr later. As shown in Fig. 4, actinomycin D inhibited carbonic anhydrase activities in kidney microsomal and

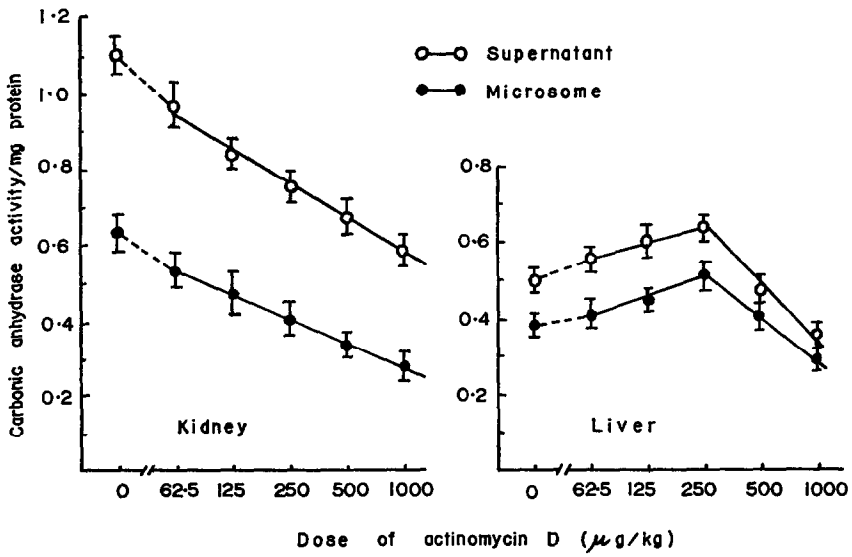

FIG. 4. Effect of various doses of actinomycin D on carbonic anhydrase from kidney and liver. Each point with vertical lines represents the mean with standard deviation.

supernatant fractions and these data using a semi-log. plot showed that a straight-line relationship existed between the depression of enzymic activity and the dose of actinomycin D. In the liver, smaller doses of actinomycin D elevated the enzymic activity in microsomal and supernatant fractions, while 500 μg/kg dose had no effect and 1000 μg/kg dose inhibited the enzymic activity in both fractions. These results agree with those obtained from mice. On the other hand, any dose of actinomycin D had no effect on total- and Mg^{2+}-ATPase activities from kidney microsomes.

Antagonism of actinomycin D to the action of aldosterone. Five animals in each group respectively were injected with 10 μg/kg of aldosterone i.p. and 3 hr later 400 μg/kg of actinomycin D was injected intraperitoneally. As shown in Fig. 5(a), the administration of actinomycin D restored the decreased kidney enzymic activity to that approaching or exceeding the control levels. In the liver, actinomycin D decreased the elevated enzymic activity to normal levels. Figure 5(c) shows the changes of total- and Mg^{+}-ATPase activities in kidney microsomes under the same conditions

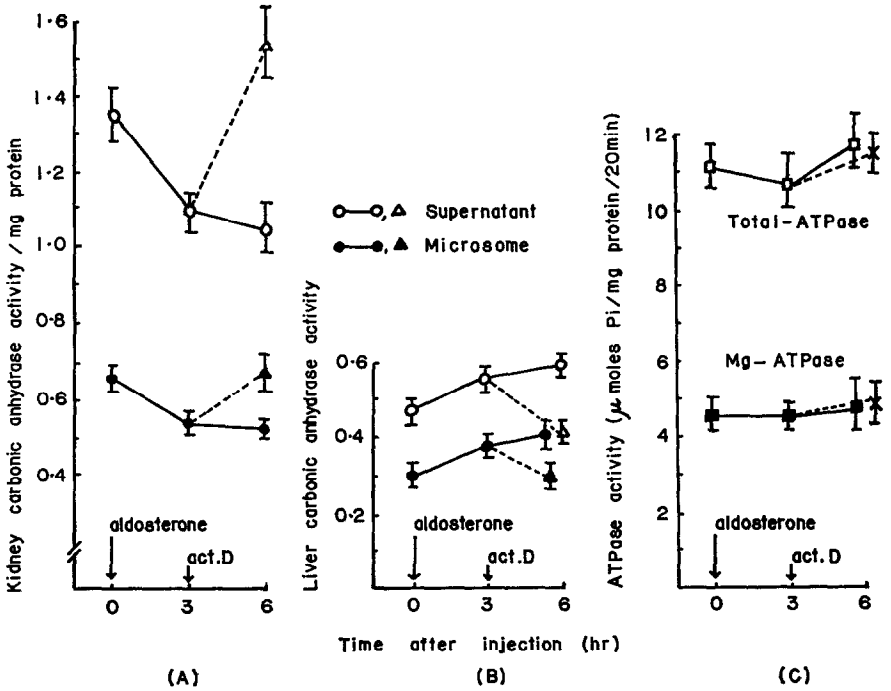

FIG. 5. Effect of aldosterone (——), alone or in combination with actinomycin D (- - - -) on carbonic anhydrase (A and B) and ATPase (C) activities. Each point with vertical lines represents the mean with standard deviation.

as above. Aldosterone and actinomycin D, alone or in combination, had no significant effect on these enzymic activities.

Experiment with adrenalectomized rats

Effect of aldosterone (*Time course experiment*). Animals adrenalectomized 5 days prior to experiment were injected i.p. with 0·5 μg/kg of aldosterone and sacrificed at various times indicated in Figs. 6 and 7. As shown in the left half of Fig. 6, carbonic anhydrase activities in kidney microsomal and supernatant fractions were elevated by adrenalectomy (0 hr values). These enzymic activities were decreased after aldosterone treatment and the maximal depression was observed 4 hr later ($P < 0{\cdot}05$). Thereafter, the enzymic activity was recovered and returned to 0 hr values by 16 hr. Liver enzymic activity was elevated by adrenalectomy and this enzymic activity was also inhibited by aldosterone treatment ($P < 0{\cdot}05$); maximal decrease was observed at 4 hr with a gradual return to 0 hr value by 16 hr. Figure 7 shows the changes of ATPase activity in the kidney microsomes. Total-ATPase activity was significantly decreased after adrenalectomy ($P < 0{\cdot}05$). Aldosterone had no effect on this enzymic activity at any time after treatment. Mg^{2+}-ATPase activity was not affected by both adrenalectomy and treatment with aldosterone at any time.

Effect of actinomycin D (*Dose–response relation*). Table 1 shows the effect of actinomycin D in doses ranging from 50 to 800 μg/kg on carbonic anhydrase and ATPase

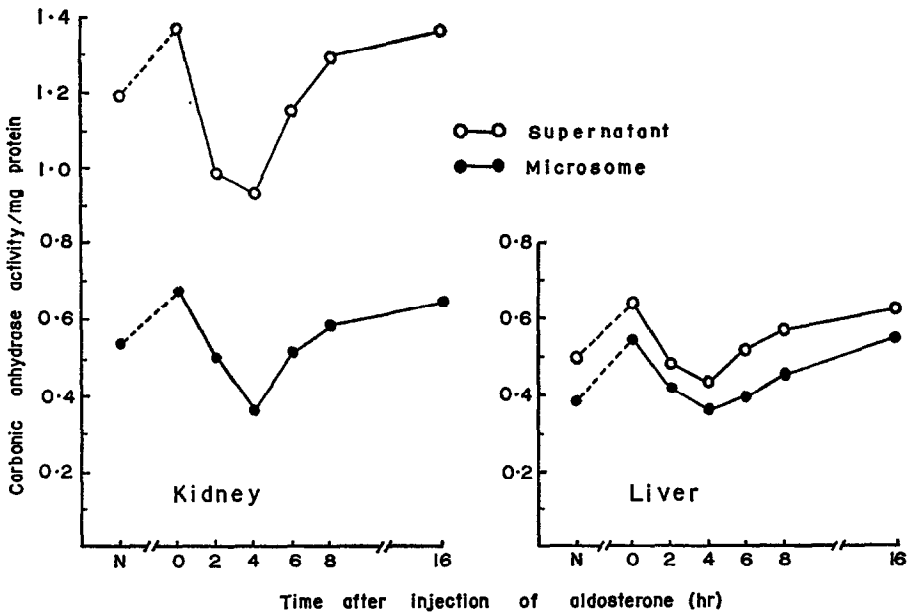

FIG. 6. Time course of response of carbonic anhydrase to aldosterone in adrenalectomized rats. Each point represents the mean from five animals. N indicates the normal values.

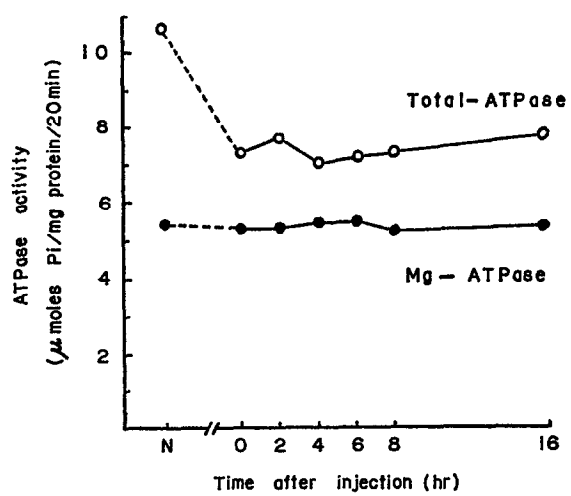

FIG. 7. Time course of response of total- and Mg^{2+}-ATPase activities to aldosterone in adrenalectomized rats. Each point represents the mean from five animals. N indicates the normal values.

activities of adrenalectomized rats at 6 hr after i.p. administration. Carbonic anhydrase activities in the kidney and liver were not affected by any dose of actinomycin D. On the other hand, total-ATPase activity in the kidney microsomes was depressed by actinomycin D, whereas Mg^{2+}-ATPase activity was not affected by any dose.

Effect of actinomycin D in aldosterone-pretreated rats. Animals were adrenalectomized 5 days prior to experiment and maintained on water and solid diet. From the

TABLE 1. DOSE–RESPONSE RELATION BETWEEN ACTINOMYCIN D AND CARBONIC ANHYDRASE AND ATPASE ACTIVITIES

Dose of Act. D	No. of rats	Carbonic anhydrase activity Enzyme unit/mg protein			Kidney microsome ATPase activity	
		Liver	Kidney			
		Supernatant	Microsomes	Supernatant	Total-ATPase	Mg^{2+}-ATPase
Normal	4	0·48 ± 0·03	0·57 ± 0·03	1·18 ± 0·03	12·88 ± 0·75	4·70 ± 0·22
Adrex.	4	0·59 ± 0·04*	0·67 ± 0·05*	1·40 ± 0·05*	9·60 ± 0·50*	4·42 ± 0·15
+ Act. D 50 μg/kg	4	0·58 ± 0·03	0·65 ± 0·03	1·36 ± 0·07	8·35 ± 0·78†	4·29 ± 0·37
+ Act. D 200 μg/kg	4	0·58 ± 0·03	0·65 ± 0·04	1·34 ± 0·06	7·77 ± 0·57†	4·32 ± 0·43
+ Act. D 800 μg/kg	4	0·59 ± 0·06	0·65 ± 0·04	1·35 ± 0·07	8·66 ± 0·10†	4·64 ± 0·34

Animals were adrenalectomized 5 days prior to experiment.
Enzymic activity represents the mean ± S.D.
* $P < 0·05$, when compared with normal group.
† $P < 0·05$, when compared with adrex. group.

third day after adrenalectomy, aldosterone in a dose of 10 μg/kg was administered s.c. once daily for 3 days. On the 6th day, animals were given an i.p. injection of actinomycin D (400 μg/kg) and sacrificed at various times indicated in Table 2. After the single i.p. injection of actinomycin D, carbonic anhydrase activities in the kidney and liver were gradually decreased and a maximal depression was observed 4–6 hr later. Then the enzymic activity was restored and returned to 0 hr values by 16 hr. On the other hand, actinomycin D inhibited total-ATPase activity indicating a minimum at 6 hr after treatment without any effect on Mg^{2+}-ATPase activity.

Effect of aldosterone and actinomycin D, alone or in combination. The present experiment was carried out according to Castles and Williamson[11] and the results are indicated in Table 3. Animals were adrenalectomized 5 days prior to experiment and one group was maintained on water and the other four groups were maintained on 0·9% saline for drinking water. In the latter four groups, one was used as control and the other three were used as experimental groups.

TABLE 2. EFFECT OF ACTINOMYCIN D ON CARBONIC ANHYDRASE AND ATPASE ACTIVITIES IN ALDOSTERONE-PRETREATED ADRENALECTOMIZED RATS

Time after injection (hr)	No. of rats	Carbonic anhydrase activity* Enzyme unit/mg protein				Kidney microsome ATPase activity*	
		Liver		Kidney			
		Microsome	Supernatant	Microsome	Supernatant	Total-ATPase	Mg^{2+}-ATPase
Normal	5	0·29	0·44	0·61	1·33	12·72	4·79
0	5	0·48	0·61	0·63	1·34	10·90	4·41
2	5	0·37†	0·56	0·57	1·15†	10·42	4·60
4	5	0·27†	0·45†	0·53†	1·06†	9·83†	4·68
6	5	0·28†	0·44†	0·54†	1·05†	8·25†	4·65
8	5	0·38	0·53	0·59	1·19†	9·64†	4·40
16	5	0·46	0·60	0·64	1·34	9·58†	4·60

* Mean from five animals.
† $P < 0·05$ (when compared with 0 hr value).

In two adrenalectomized groups given water and saline (groups 2 and 3), kidney and liver carbonic anhydrase activities were elevated after adrenalectomy with no differences between them. In aldosterone (0·34 μg/kg) alone treated group, carbonic anhydrase activities in microsomal and supernatant fractions from kidney and liver were decreased and approached the normal values 4 hr later. Especially, the enzymic activity in the kidney supernatant fraction was depressed under normal levels. Whereas actinomycin D had no effect on the enzymic activity by itself but blocked the inhibitory effect of aldosterone when it was administered i.p. 1 hr prior to aldosterone. Total-ATPase activity in adrenalectomized and water given group was decreased to about 68 per cent of normal value. Whereas the decrease of this enzymic activity in adrenalectomized and saline given group was about 10 per cent of the normal value. The administration of aldosterone and actinomycin D, alone or in combination, had no significant effect on ATPase activity.

TABLE 3. EFFECT OF ALDOSTERONE AND ACTINOMYCIN D, ALONE OR IN COMBINATION, ON CARBONIC ANHYDRASE AND ATPASE ACTIVITIES

Group and treatment	No. of rats	Carbonic anhydrase activity: Mean ± S.D. Enzyme unit/mg protein				Kidney microsome ATPase activity (μM P_i/mg protein/20 min)
		Liver		Kidney		
		Microsomes	Supernatant	Microsomes	Supernatant	
1. Normal (water)	5	0·39 ± 0·02	0·53 ± 0·03	0·59 ± 0·02	1·28 ± 0·04	11·73 ± 0·48
2. Adrex. (water)	5	0·59 ± 0·02*	0·64 ± 0·03*	0·74 ± 0·03*	1·46 ± 0·04*	8·00 ± 0·53*
3. Adrex. (saline)	5	0·60 ± 0·03*	0·64 ± 0·02*	0·73 ± 0·02*	1·48 ± 0·07*	10·49 ± 0·43*
4. Adrex. (saline) + aldost. 0·34 μg/kg	5	0·47 ± 0·02†	0·52 ± 0·02†	0·56 ± 0·03†	1·09 ± 0·05†	10·65 ± 0·40
5. Adrex. (saline) + act. D 400 μg/kg	5	0·61 ± 0·02	0·63 ± 0·02	0·72 ± 0·02	1·45 ± 0·09	10·83 ± 0·51
6. Adrex. (saline) + act. D 400 μg/kg aldost. 0·34 μg/kg	5	0·59 ± 0·03	0·62 ± 0·03	0·71 ± 0·02	1·52 ± 0·05	11·21 ± 0·45

In group 4, animals were injected s.c. with aldosterone 4 hr before sacrifice.
In group 5, animals were injected i.p. with actinomycin D 5 hr before sacrifice.
In group 6, actinomycin D was injected 1 hr before aldosterone treatment and sacrificed 5 hr later.
* $P<0·05$, when compared with normal group.
† $P<0·05$, when compared with group 3.

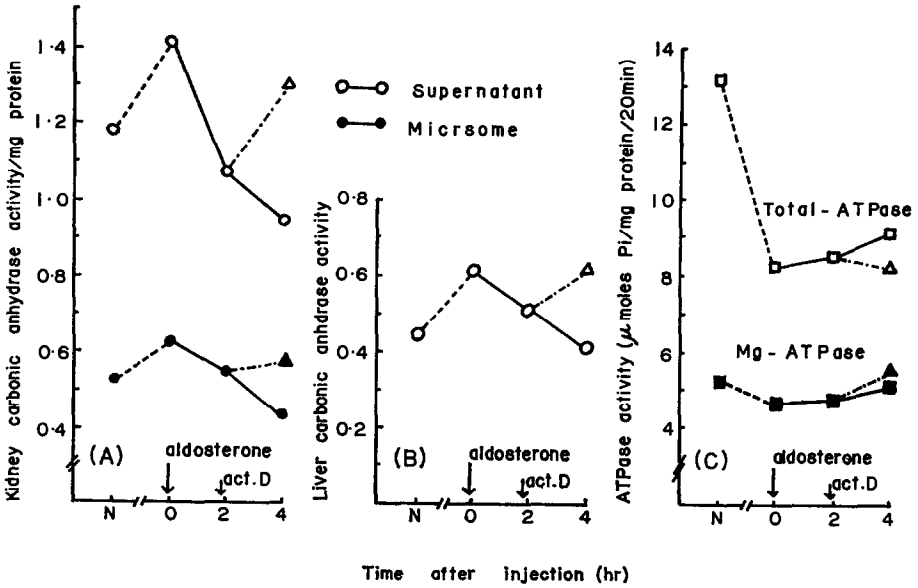

FIG. 8. Antagonistic effect of actinomycin D to the action of aldosterone on carbonic anhydrase and ATPase activities. Each point represents the mean from four animals. N indicates the normal values. Aldosterone (——), Actinomycin D (– · – · –).

Antagonism of actinomycin D to the action of aldosterone. Animals consisted of four rats in each group were adrenalectomized 5 days prior to experiment and maintained on water and solid diet. On the 6th day after adrenalectomy, animals were injected i.p. with 10 μg/kg of aldosterone and sacrificed 2 and 4 hr later. In one group, animals were injected i.p. with 400 μg/kg of actinomycin D at 2 hr after aldosterone treatment and sacrificed 2 hr later. As shown in Fig. 8(a), carbonic anhydrase activities in the kidney microsomal and supernatant fractions were decreased after aldosterone ($P < 0{\cdot}05$). Whereas, actinomycin D administered at 2 hr after aldosterone restored the decreased enzymic activity to control levels. Figure 8(b) shows the changes of liver enzymic activity. Aldosterone depressed carbonic anhydrase activity in supernatant fraction and combined administration of actinomycin D restored the decreased enzymic activity to control level. Figure 8(c) shows the changes of total- and Mg^{2+}-ATPase activities in kidney microsomes. Aldosterone and actinomycin D had no significant effect on both enzymic activities.

DISCUSSION

It has been reported that the minimum detectable dose of aldosterone measured by Na/K concentration ratio in urine or urinary sodium excretion is from 0·03 to 0·12 μg in adrenalectomized rats.[7] Further, the alterations of urinary Na and K excretions appear in 2–4 hr after treatment of aldosterone in adrenalectomized rats[11,12] and man.[21] As shown in Fig. 1, maximal response of kidney carbonic anhydrase to aldosterone appeared 2–4 hr after treatment. In view of these observations, we could conclude that kidney carbonic anhydrase is very sensitive to aldosterone and the changes of this enzymic activity might have a close relation with the physiological action of aldosterone.

Aldosterone seems to act as a repressor on carbonic anhydrase of rat kidney. As kidney carbonic anhydrase is insensitive to glucocorticoids,[22] the elevation of this enzymic activity after adrenalectomy may be due to the lack of aldosterone. On the other hand, since the liver carbonic anhydrase activity is controlled by both glucocorticoids[22] and aldosterone; essential effect of the former is inhibitory and that of the latter is stimulatory, it may be considered in normal rats that the administration of aldosterone results in a temporary disturbance on the balance between glucocorticoid and mineralocorticoid and stimulates liver enzymic activity. Presumably, liver enzymic activity may be more sensitive to glucocorticoid and adrenalectomy may cause an elevation of this enzymic activity. However, the mechanism of inhibitory action of aldosterone on liver enzymic activity in adrenalectomized rats is not clear (Fig. 6).

Recently, it has been reported that aldosterone exerts the effect through the synthesis of RNA and proteins which in turn are responsible for the physiological action of the hormone in toad bladder,[13,23] rat kidney[11,12,24] and rabbit kidney.[25] Castles and Williamson[11] and Fimognari *et al.*[12] also have reported that actinomycin D inhibited the decrease of urinary excretion of sodium caused by aldosterone in adrenalectomized rats. Actinomycin D is considered to bind with template DNA and interfere with the synthesis of RNA by DNA-dependent RNA polymerase.[26,27] In the present experiment, however, it is not clear that the effect of aldosterone and actinomycin D on kidney carbonic anhydrase system is due to whether the control of this enzyme synthesis or inactivation of enzymic activity. According to our unpublished data, aldosterone and actinomycin D inhibit carbonic anhydrase activity of rat kidney homogenate without any direct effect on the enzymic activity of microsomal and supernatant fractions *in vitro*. The presence of nuclei in the reaction system seems to be necessary for the appearance of the effect of aldosterone and actinomycin D on carbonic anhydrase activity.

Further, aldosterone and actinomycin D alone depressed kidney carbonic anhydrase activity; an antagonism was observed when these drugs were administered in combination (Figs. 5 and 8). These phenomena seem to offer an important problem as to the mechanism of action of aldosterone and actinomycin D on carbonic anhydrase system. However, no definite explanation on this mechanism can be offered at present.

It was interesting to note that the effect of actinomycin D on kidney carbonic anhydrase activity could not be observed in adrenalectomized rats (Table 1); however, in aldosterone-pretreated adrenalectomized rats, carbonic anhydrase activity was again inhibited by actinomycin D as in normal rats (Table 2). Although the fine mechanism of this phenomenon is not clear, the sensitivity of kidney cells to actinomycin D may be recovered by aldosterone. That is, aldosterone has a trigger-like property to actinomycin D. Similar phenomenon has been reported by Goodman *et al.*[28] that ouabain causes a significant inhibition on sodium transport in aldosterone-treated toad bladder sections, but has no effect in comparable control sections.

Chignell and Titus[29] and Landon *et al.*[30] have reported that the single administration of aldosterone to adrenalectomized rats *in vivo* produces no detectable changes in (Na^+K^+)- and Mg^{2+}-ATPase activities after 3 hr, even though the maximal sodium retention after aldosterone treatment may be observed at that time. In the present experiment, total- and Mg^{2+}-ATPase activities were not affected by aldosterone in normal and adrenalectomized rats. Kidney ATPase system may be independent of aldosterone.

On the other hand, actinomycin D had no effect on ATPase activity in normal rats, while it inhibited total-ATPase activity of adrenalectomized rats excepting in the case of Table 3. However, we cannot offer an adequate explanation on the inhibitory effect of actinomycin D in adrenalectomized rats. Presumably, the sensitivity of kidney cells to actinomycin D may alter after adrenalectomy.

Recently, Jørgensen[31] has offered the following consideration with adrenalectomized rats that an elevation in kidney ATPase activity after repeated administration of aldosterone is not due to a primary action of this hormone but a secondary effect by other events, e.g. alterations of Na and K concentration in plasma and aldosterone could not seem to be the regulating factor on the maintenance of kidney ATPase activity. Further, in the next[32] he reported that the reaction of kidney ATPase from adrenalectomized rats to aldosterone was different in three sub-divisions; outer medulla, inner medulla and outer cortex, and ATPase activity from outer medulla was elevated by aldosterone, while the enzymic activity in the latter two was insensitive to aldosterone. Our kidney samples were mainly obtained from cortex. This may be the reason that ATPase activity is not affected by aldosterone in the present experiment. Alterations of kidney ATPase activity after aldosterone treatment have not been investigated with normal rats.

In the previous reports,[8,9] we observed the stimulatory effect of aldosterone on carbonic anhydrase activity from mouse kidney. In the present experiment, aldosterone inhibited kidney carbonic anhydrase activity in rats. At the present time, no explanation for the species-difference between mice and rats can be offered. However, similar differences also have been reported with the effect of oestradiol and ovariectomy on uterine carbonic anhydrase activity of mice[33–35] and rats.[36,37]

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