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REGULATION OF THE ($Na^+ + K^+$)-ACTIVATED ATP HYDROLYZING ENZYME SYSTEM IN RAT KIDNEY. I. THE EFFECT OF ADRENALECTOMY AND THE SUPPLY OF SODIUM ON THE ENZYME SYSTEM

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SUMMARY

1. Activity of the ($Na^+ + K^+$)-activated ATP hydrolyzing enzyme was measured in the subcellular fractions from normal and adrenalectomized rat kidneys before and after incubation with sodium deoxycholate.

2. By incubation with deoxycholate + EDTA activity of the ($Na^+ + K^+$)-activated enzyme increased about 3-fold, while the activity with Mg^{2+} alone remained unchanged. Using this procedure total activity of the ($Na^+ + K^+$)- activated enzyme could be measured in a reproducible way.

3. A 35 to 46% reduction in activity of the ($Na^+ + K^+$)-activated enzyme was observed after adrenalectomy in preparations treated with deoxycholate + EDTA, while the changes in the fresh preparations were small and located in the microsomal fractions. Thus part of the decrease in activity could be ascribed to a change in response to deoxycholate of the enzyme in kidneys from adrenalectomized rats.

4. The decrease in amount of the ($Na^+ + K^+$)-activated enzyme during developing adrenal insufficiency corresponded to the rate of change of plasma concentrations of sodium and potassium.

5. A high sodium diet could postpone and partly prevent the decrease in activity of the enzyme during developing adrenal insufficiency. This finding suggests that the enzyme is not primarily under the control of the adrenals, and might indicate an effect of sodium on activity levels of the ($Na^+ + K^+$)-activated enzyme in rat kidney.

INTRODUCTION

Considerable evidence suggests that the ($Na^+ + K^+$)-activated ATP hydrolyzing enzyme system, isolated from a large number of tissues including kidney[2-4], is involved in the active transport of Na^+ and K^+ (see ref. 1). Activity of this enzyme prepared from kidney is reduced after adrenalectomy in rats[5,6], but a direct relationship between mineralocorticoid administration and activity of the enzyme has not been established.

However, the findings suggest that alterations in activity of the ouabain-sensitive $(Na^+ + K^+)$-activated enzyme can play a role in the regulation of renal excretion of cations, and information on the factors which regulate the enzyme and on the molecular basis for the changes in activity is needed.

It is possible by incubation of the fresh subcellular fractions from kidney with deoxycholate to increase the activity of the $(Na^+ + K^+)$-activated enzyme several times (J. C. SKOU, personal communication), while the presence of the detergent in excess is inhibitory[2]. The results of earlier investigators show that activity of the enzyme in rat kidney is dependent on the method of preparation used[3,6,7].

It was therefore considered of primary importance to define the conditions for quantitative measurement of this enzyme in rat kidney and to study its distribution in kidneys from normal and adrenalectomized rats under conditions where actual total activity[8] of the enzyme is measured.

In an attempt to relate activity of the enzyme to the changes in ionic balance after adrenalectomy the influence of sodium intake on the decrease in activity during developing adrenal insufficiency was studied and compared with the changes in plasma electrolyte concentrations.

METHODS

Male Wistar rats weighing from 200 to 250 g and kept in a constant-temperature room at 26 $\pm$ 1° were used. The diet consisted of Altromin pellets given *ad libitum*.

If not otherwise specified, adrenalectomized rats had free access to both tap-water and 0.9% NaCl solution. Adrenalectomy was performed by lumbar approach under Mebumal sodium anaesthesia, 35 mg/kg rat. Removal of both adrenals with intact capsule was required.

The adrenalectomized rats were used 7 to 9 days after the operation. The changes in plasma electrolyte concentrations were considered the most reliable criteria of adrenal insufficiency[9] and a plasma Na^+/K^+ ratio below 30 (26.4 $\pm$ 0.9, normal rats 39.7 $\pm$ 0.8) was required. The adrenalectomized rats had a higher plasma K^+ concn. (5.3 $\pm$ 0.2 mM) than normal rats (3.6 $\pm$ 0.1 mM) and a slightly lower plasma Na^+ concn. (139 $\pm$ 0.8 mM, normal 142 $\pm$ 0.6 mM).

Tissue preparation. At the time of sacrifice the rats were anaesthetized with ether. Blood samples for plasma Na^+ and K^+ measurements were taken from the inferior vena cava in a syringe containing 25 I.U. heparin, and the rats were killed by exsanguination from the aorta. The kidneys were removed and immediately placed in ice-cold 0.25 M sucrose, 0.03 M histidine (pH 7.2, 20°). After cooling they were decapsulated and weighed on a torsion balance. Only one kidney (the left) were used for preparation, since the material obtained in each of the sediments from one kidney was sufficient for several enzyme assays.

The kidney was homogenized in a Potter–Elvehjem Teflon-glass homogenizer with 5 ml ice-cold 0.25 M sucrose, 0.03 M histidine (pH 7.2 with HCl).

The homogenate was conveyed to 13-mm cellulose-nitrate tubes and fractionated by differential centrifugation at 0° in a Serwall angle centrifuge. Centrifugation at 105 000 $\times$ g was performed in a Christ, model Omega, ultracentrifuge.

The following fractions were collected: Sed_{1500}, sediment after 1500 $\times$ g for 15 min; $Sed_{10\,800}$, sediment after 10 800 $\times$ g for 30 min; $Sed_{25\,300}$, sediment after

25 300 × *g* for 30 min; $Sed_{105\,000}$, sediment after 105 000 × *g* for 60 min; $Sup_{105\,000}$, supernatant from $Sed_{105\,000}$. After the second centrifugation, 3 ml 0.25 M sucrose, 0.03 M histidine were added to the supernatant.

In the initial experiments each of the sediments was resuspended in the original volume of 5 ml 0.25 M sucrose, 0.03 M histidine (pH 7.2) and stored at −20°. Later only $Sed_{25\,300}$ was stored and the other sediments discarded.

The chief aim was to obtain reproducible preparations of the 'heavy' microsomal fraction ($Sed_{25\,300}$) after sedimentation of the mitochondria at 10 800 × *g* for 30 min (see ref. 10). The moderate variations in the concentration of protein in the resuspended $Sed_{25\,300}$ (the coefficient of variation calculated from the values in Table IV was 9% and 11%) show that it was possible to keep the conditions of centrifugation constant from time to time.

Enzyme assay. Enzyme activity was determined as described by SKOU[11]. The reaction was started by addition of enzyme in appropriate dilution to split 10 to 15% of the total ATP present (3 mM) in 15 min at 37°. Under these conditions, ATP hydrolysis was a linear function of time and of enzyme concentration.

Activity units. Specific activity was expressed as μM P_i per mg protein per h and activity in whole kidney as μM P_i per mg kidney per h.

Incubation with deoxycholate and EDTA (see ref. 12). The sediment was incubated at 0° with the concentrations of sodium deoxycholate and EDTA which gave maximum enhancement of the $(Na^+ + K^+)$-activated enzyme without noticeable change in the activity with Mg^{2+} alone. The medium which proved to be optimal for $Sed_{25\,300}$ from both normal and adrenalectomized rats contained 2.4 mM sodium deoxycholate, 3 mM EDTA, 50 mM imidazole (pH 7.5 at 20°); pH was controlled after the incubation.

The incubation was started by addition of a sufficient quantity of enzyme, *e.g.* 0.4 to 0.5 mg protein per ml incubation medium for $Sed_{25\,300}$. At various times 50 μl were transferred to the test tubes and enzyme activity determined as described above.

Sodium and potassium determination. Sodium and potassium in urine and serum were determined by flame photometry (Eppendorf). pH was measured with a Radiometer capillary glass electrode.

Protein assay. Protein was measured by the method of LOWRY *et al.*[12] after precipitation with trichloroacetic acid and NaOH hydrolysis. The method was standardized with micro-Kjeldahl determinations on the enzyme preparations and bovine serum albumin used as working standard.

Expression of results. Mean values ± S.E. (standard error of the mean) are given. Significance of differences between means was calculated by Students' t test.

RESULTS

The effect of incubation with deoxycholate + EDTA on the $(Na^+ + K^+)$-activated and the ouabain-insensitive Mg^{2+}-activated ATP hydrolyzing enzymes is shown in Fig. 1. A rapid change in activity of the $(Na^+ + K^+)$-activated enzyme was observed and a constant activity reached after incubation for 20 min, without noticeable change in the ouabain-insensitive activity. The level of the control, in-

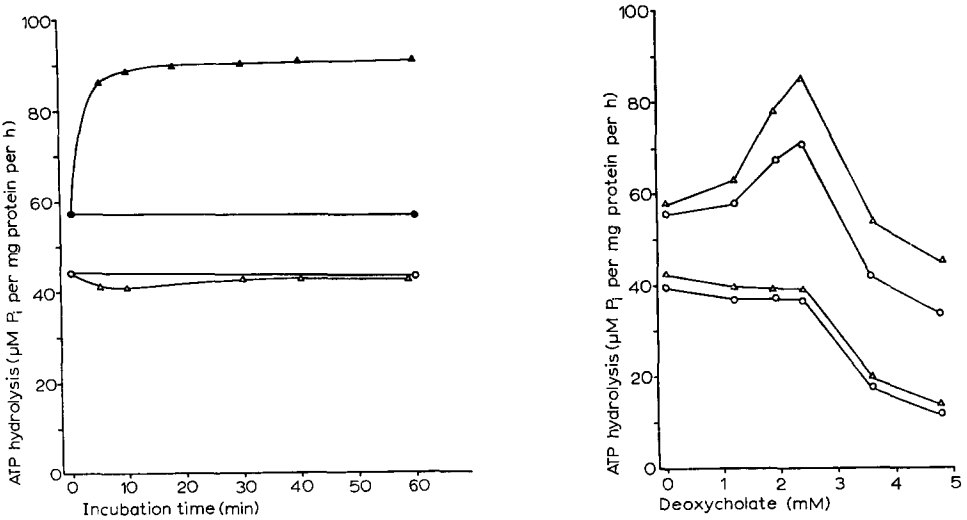

Fig. 1. The rate of increase in activity of the $(Na^+ + K^+)$-activated enzyme in $Sed_{25\ 300}$ from normal rat kidney during incubation at 0° with 2.4 mM deoxycholate, 3 mM EDTA, 50 mM imidazole (pH 7.5, 20°). Test solutions contained 3 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+ with and without 1 mM ouabain, 0.03 M histidine (pH 7.5, 37°). ATP concn., 3 mM. ▲, activity with Mg^{2+}–Na^+–K^+ after incubation with deoxycholate + EDTA; △, activity with Mg^{2+}–Na^+–K^+ + 1 mM ouabain after incubation with deoxycholate + EDTA; ●, activity of the control with Mg^{2+}–Na^+–K^+ after incubation without deoxycholate + EDTA; ○, activity of the control with Mg^{2+}–Na^+–K^+ + 1 mM ouabain.

Fig. 2. Effect of incubation for 30 min at 0° with varying deoxycholate concns. on the activity in $Sed_{25\ 300}$ from normal (△) and adrenalectomized (○) rat kidneys. Except for the variation in deoxycholate concn. experimental conditions were as for Fig. 1.

cubated without deoxycholate + EDTA was unaffected. Incubation with EDTA alone had no effect on activity of the enzymes.

Fig. 2 shows that the effect of deoxycholate was concentration-dependent and that the enhancement in activity obtained by increasing the deoxycholate concentration was greater with $Sed_{25\ 300}$ from normal than from adrenalectomized rat kidney (*cf.* Table IV). Maximum activity of the $(Na^+ + K^+)$-activated enzyme was acquired with 2.4 mM deoxycholate, and at this concentration inhibition of the ouabain-insensitive activity was less than 10%. Moreover it is apparent that the decrease in activity caused by high deoxycholate concentrations was mainly due to inhibition of the ouabain-insensitive, Mg^{2+}-activated enzyme. Thus the constancy of this activity could be used to ascertain that enzyme destruction had not taken place and provided a basis from which the $(Na^+ + K^+)$-activated enzyme could be measured.

At pH 7.5 the optimum EDTA concentration was 3 mM. A further increase in the concentration of EDTA in the incubation medium to 6 and 9 mM gave a gradual decrease in ouabain-sensitive activity of preparations from both normal and adrenalectomized rat kidneys and, contrary to the findings of LANDON, JAZAB AND FORTE[5], EDTA did not eliminate the difference in activity between these preparations. EDTA was not necessary for the activation produced by deoxycholate (J. C. SKOU, personal communication), but without EDTA in the incubation medium the enzyme was more labile and the results less consistent.

TABLE I

ACTIVITY OF ATP HYDROLYZING ENZYMES IN SUBCELLULAR FRACTIONS OF FRESH KIDNEY PREPARATIONS

Activity with 3 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, ± 1 mM ouabain in the fresh (*i.e.* not deoxycholate-treated) preparations from 4 normal (N) and 4 adrenalectomized (A) rats. Total activity per mg kidney was defined as the sum of the activities per mg kidney in the subcellular fractions. Ouabain-sensitive activity is given both as activity per mg kidney and as activity per mg protein and was calculated as the difference in activity with and without 1 mM ouabain. The test solutions contained 3 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, 3 mM ATP, 30 mM histidine (pH 7.5), with or without 1 mM ouabain in a total vol. of 1 ml.

Fraction	*Number of rats*	*μM P_i per h per mg kidney*				*μM P_i per h per mg protein*		
		Mg^{2+}–Na^+–K^+	*Mg^{2+}–Na^+–K^+ 1 mM ouabain*	*Sensitive to 1 mM ouabain*	*Difference N − A* N (%)	*Sensitive to 1 mM ouabain*	*Difference N − A* N (%)	*P*
Sed_{1500}	N 4	2.38 ± 0.03	1.99 ± 0.03	0.39 ± 0.05	5	6.7 ± 1.1	9	
	A 4	2.47 ± 0.11	2.10 ± 0.14	0.37 ± 0.04		6.1 ± 0.6		
$Sed_{10\ 800}$	N 4	0.83 ± 0.06	0.64 ± 0.05	0.20 ± 0.02	3	12.9 ± 1.2	11	
	A 4	0.90 ± 0.02	0.69 ± 0.02	0.21 ± 0.02		11.4 ± 0.4		
$Sed_{25\ 300}$	N 4	0.83 ± 0.02	0.61 ± 0.03	0.22 ± 0.02	31	16.9 ± 0.5	23	<0.005
	A 4	0.69 ± 0.02	0.55 ± 0.02	0.15 ± 0.02		13.0 ± 0.5		
$Sed_{105\ 000}$	N 4	0.50 ± 0.01	0.46 ± 0.01	0.04 ± 0.01	68	4.8 ± 0.1	69	<0.001
	A 4	0.47 ± 0.03	0.47 ± 0.03	0.013 ± 0.01		1.5 ± 0.5		
$Sup_{105\ 000}$	N 4	0.09 ± 0.02	0.08 ± 0.02	0.004				
	A 4	0.06 ± 0.01	0.06 ± 0.01	0.004				
Total	N 4	4.63 ± 0.07	3.78 ± 0.07	0.84 ± 0.07	12			
	A 4	4.60 ± 0.12	3.85 ± 0.13	0.74 ± 0.05				

Activity of the ATP hydrolyzing enzymes was measured in each of the subcellular fractions in the fresh state (Table I) and after preincubation with deoxycholate + EDTA (Table II). The (Na^+ + K^+)-activated enzyme was found in all fractions, but the data were not conclusive with respect to the subcellular distribution of the enzyme, since the fractions were not further purified.

It is seen from the values in Tables I and II that the subcellular fractions could be prepared by differential centrifugation with an accuracy which allowed a comparison between preparations from normal and adrenalectomized rat kidney of both specific and absolute activities of the ATP hydrolyzing enzymes.

In the fresh preparations (Table I) over 80% of the activity was ouabain-insensitive. The amount of this enzyme was the same in both normal and adrenalectomized rat kidneys. In the fresh microsomal fractions, $Sed_{25\ 300}$ and $Sed_{105\ 000}$, a significant decrease was observed in the ouabain-sensitive, (Na^+ + K^+)-activated enzyme after adrenalectomy, while the differences were small and insignificant in Sed_{1500}, $Sed_{10\ 800}$ and in the calculation of total activity in the kidneys.

In Table II is shown the activity in the same sediments as in Table I, but after incubation of each of the subcellular fractions with deoxycholate + EDTA. A comparison between the figures in Tables I and II shows that deoxycholate +

TABLE II

ACTIVITY OF ATP HYDROLYZING ENZYMES IN SUBCELLULAR FRACTIONS OF KIDNEY PREINCUBATED WITH DEOXYCHOLATE AND EDTA

Activity of the preparations analyzed in Table I after incubation with 2.4 mM deoxycholate, 3 mM EDTA, 50 mM imidazole (pH 7.5) at 0° for 30 min. Test solutions as described for Table I.

Fraction	*Number of rats*	*μM P_i per h per mg kidney*				*μM P_i per h per mg protein*		
		Mg^{2+}–Na^+–K^+	*Mg^{2+}–Na^+–K^+ 1 mM ouabain*	*Sensitive to 1 mM ouabain*	*Difference N − A* / *N (%)*	*Sensitive to 1 mM ouabain*	*Difference N − A* / *N (%)*	*P*
Sed_{1500}	N 4	3.17 ± 0.13	2.31 ± 0.13	0.87 ± 0.10		15.3 ± 1.6		
					37		39	<0.05
	A 4	3.09 ± 0.15	2.54 ± 0.13	0.55 ± 0.10		9.3 ± 1.7		
$Sed_{10\ 800}$	N 4	1.18 ± 0.11	0.73 ± 0.07	0.45 ± 0.04		31.0 ± 3.7		
					18		37	<0.05
	A 4	1.15 ± 0.02	0.79 ± 0.02	0.37 ± 0.01		19.6 ± 0.9		
$Sed_{25\ 300}$	N 4	1.20 ± 0.04	0.59 ± 0.03	0.61 ± 0.02		48.6 ± 1.4		
					41		35	<0.001
	A 4	0.89 ± 0.02	0.53 ± 0.02	0.36 ± 0.02		31.6 ± 1.4		
$Sed_{105\ 000}$	N 4	0.62 ± 0.01	0.41 ± 0.02	0.22 ± 0.02		26.1 ± 2.6		
					50		53	<0.005
	A 4	0.51 ± 0.04	0.40 ± 0.03	0.11 ± 0.01		12.3 ± 1.5		
$Sup_{105\ 000}$	N 4	0.09 ± 0.02	0.08 ± 0.02					
	A 4	0.06 ± 0.01	0.06 ± 0.01					
Total	N 4	6.17 ± 0.14	4.02 ± 0.21	2.15 ± 0.21				
					38			<0.01
	A 4	5.64 ± 0.12	4.30 ± 0.11	1.34 ± 0.12				

EDTA caused an increase in activity with 3 mM Mg^{2+}, 100 mM Na^+ and 20 mM K^+ due mainly to an increase in the ouabain-sensitive, $(Na^+ + K^+)$-activited ATP hydrolysis. This increase in the ouabain-sensitive activity was greater for the sediments from normal than from adrenalectomized rat kidneys and thus the incubation with deoxycholate + EDTA caused an increase in the per cent differences between the ouabain-sensitive activities of the two groups. After incubation with deoxycholate + EDTA, these differences became significant for all the subcellular fractions and in the calculation of total activity per mg kidney. The ouabain-insensitive, Mg^{2+}-activated ATP hydrolysis remained constant during incubation of $Sed_{25\ 300}$ and $Sed_{105\ 000}$ with deoxycholate + EDTA, while enhancement of this activity was observed in Sed_{1500} and $Sed_{10\ 800}$, probably due to release of the mitochondrial Mg^{2+}-activated ATPase[13].

The change in activity of the $(Na^+ + K^+)$-activated enzyme observed after adrenalectomy was about the same whether the activity was expressed per mg kidney or per mg protein. This agrees with the observation that no significant changes occurred in water content or weight of the kidney after adrenalectomy[6] (Table III). The results in Table II show that $Sed_{25\ 300}$, where the highest specific activity of the enzyme was found, was a good representative for the general changes in enzyme activity found in whole kidney. The proportion of the total amount of $(Na^+ + K^+)$-activated enzyme deposited in this fraction was 28% for both groups.

TABLE III

WEIGHT AND WATER CONTENT OF KIDNEYS FROM NORMAL AND ADRENALECTOMIZED RATS

Total kidney weight (right + left) in per cent of body weight and water content of kidneys from normal and adrenalectomized rats. Water content was determined by drying the minced tissue for 5 days at 105°. Values are mean ± S.E. Numbers in parenthesis are numbers of rats used.

Rat kidney	*Body weight range (g)*	*Kidney weight (% body weight)*	*Kidney water content (mg water per g wet tissue)*
Normal	197–258	0.72 ± 0.01 (20)	765 ± 1.1 (9)
Adrenalectomized	200–260	0.71 ± 0.02 (15)	758 ± 4.8 (7)

To evaluate the optimal conditions for the quantitative estimation of enzyme activity, the general kinetic properties of the ($Na^+ + K^+$)-activated enzyme in $Sed_{25\ 300}$ were investigated and found unchanged after adrenalectomy.

In assays with varying ATP concentrations over the range of 0.3 to 6 mM and at a constant Mg^{2+} to ATP ratio of 1:1, the apparent K_m calculated for the total ATP concentration from a double reciprocal plot was 0.22 mM for normal and 0.20 mM for adrenalectomized rat kidney preparations and thus far below the concentrations used in the assays (3 mM).

Fig. 3 shows that pH optimum for the activation by $Na^+ + K^+$ was 7.5 with a rather flat maximum between 7.3 and 7.7 for both preparations.

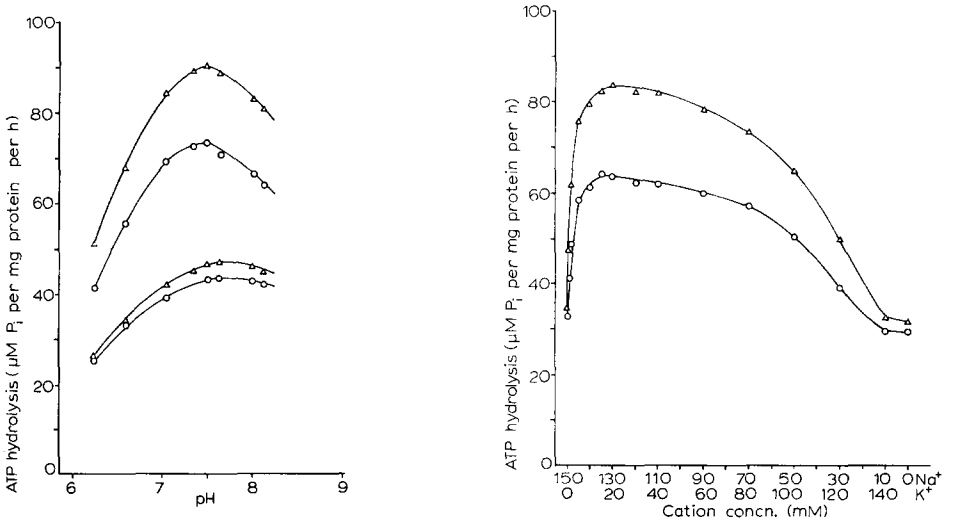

Fig. 3. Relation between pH and enzyme activity in $Sed_{25\ 300}$ from normal (△) and adrenalectomized (○) rat kidneys. Incubated for 30 min in the medium described for Fig. 1. Test solutions contained 3 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, 3 mM ATP, 50 mM imidazole (pH 6.25–8.12, 37°) with or without 1 mM ouabain. pH was measured at 37° in parallel prepared test solutions.

Fig. 4. Effect of $Na^+ + K^+$ on ATP hydrolysis in $Sed_{25\ 300}$ from normal (△) and adrenalectomized (○) rat kidney. Incubated for 30 min in the medium described for Fig. 1. The sum of $Na^+ + K^+$ was kept constant at 150 mM. 3 mM Mg^{2+}, 3 mM ATP (pH 7.5).

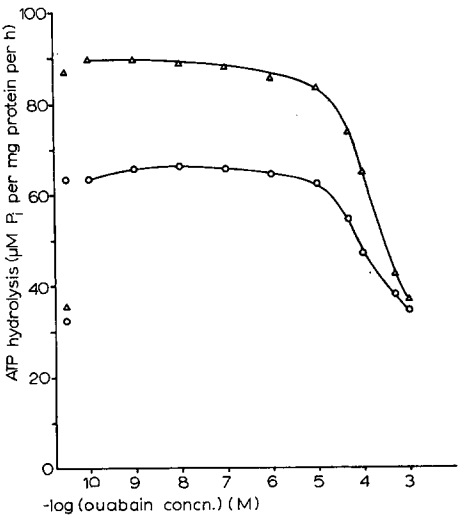

Fig. 5. Effect of ouabain on the $(Na^+ + K^+)$-activated enzyme in $Sed_{25\ 300}$ from normal (△) and adrenalectomized (○) rat kidney. Incubated for 30 min in the medium described for Fig. 1. The test solutions contained 3 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, 3 mM ATP and ouabain in the concns.shown on the abscissa, pH 7.5. To the left is shown the activity with 3 mM Mg^{2+} alone and Mg^{2+}–Na^+–K^+ buffer without ouabain.

The differences in the activating effect of $Na^+ + K^+$ is shown in Fig. 4 where the ratio between Na^+ and K^+ was varied at a constant ionic strength. The per cent difference in activity between the two enzymes was about the same for all ratios of

TABLE IV

AVERAGE PROTEIN CONCENTRATION AND SPECIFIC ACTIVITY OF ATP HYDROLYZING ENZYMES IN FRESH AND DEOXYCHOLATE-TREATED $SED_{25\ 300}$

Kidneys of 14 normal and 14 adrenalectomized rats were used. The value for each sediment was the average of two assays performed 30 and 60 min after the start of the incubation with deoxycholate + EDTA. Incubation medium and test solutions as for Fig. 1. Activity under 'fresh' was measured after parallel control incubations without deoxycholate and EDTA.

Rat kidney	*Number of rats*	*mg protein per ml*	*μM P_i per mg protein per h*			*Difference N − A* / *A (%)*	*P*
			Mg^{2+}–Na^+–K^+	*Mg^{2+}–Na^+–K^+ + 1 mM ouabain*	*Sensitive to 1 mM ouabain*		
Normal*	14	2.22 ± 0.04	59.1 ± 1.3	43.6 ± 1.3	15.5 ± 0.6	23	<0.001
Adrenalectomized*	14	2.08 ± 0.06	55.2 ± 1.1	43.2 ± 1.0	12.0 ± 0.5		
Normal**	14		87.6 ± 1.3	42.0 ± 1.1	45.6 ± 1.0	35	<0.001
Adrenalectomized**	14		70.0 ± 0.9	40.2 ± 0.9	29.8 ± 0.7		

* Fresh.
** Preincubated with deoxycholate *plus* EDTA.

Na^+ to K^+ and the optimal Na^+/K^+ ratio between 5 to 1 and 9 to 1 for both enzymes. Only slight differences in activity were found with Mg^{2+} *plus* either Na^+ or K^+ in the medium.

Fig. 5 shows that the concentration of ouabain required for half maximum inhibition of the activity due to the combined effect of $Na^+ + K^+$ was 0.1 mM for both preparations and that the level of activity with Mg^{2+} alone was reached with 1 mM ouabain. This relative insensitivity to ouabain is in agreement with earlier observations[3,7].

Table IV shows the specific activity in $Sed_{25\ 300}$ prepared from 14 normal rats and 14 adrenalectomized rats killed 7 to 9 days after the operation. These rats belonged to 5 consecutive experimental groups, but the specific activity of the ($Na^+ + K^+$)-activated enzyme after preincubation with deoxycholate + EDTA was remarkably constant from time to time and overlap between values for adrenalectomized and normal rats was not observed. The decrease in specific activity found in both fresh and deoxycholate-treated microsomes after adrenalectomy was confined to the ouabain-sensitive activity, while the ouabain-insensitive activity, equal to the activity with Mg^{2+} alone, was essentially the same. By preincubation with 2.4 mM deoxycholate + 3 mM EDTA the activating effect of $Na^+ + K^+$ was increased 2.9-fold in normal and 2.5-fold in adrenalectomized rat kidney preparations, indicating a difference in the reaction to deoxycholate whereby the change in activity after

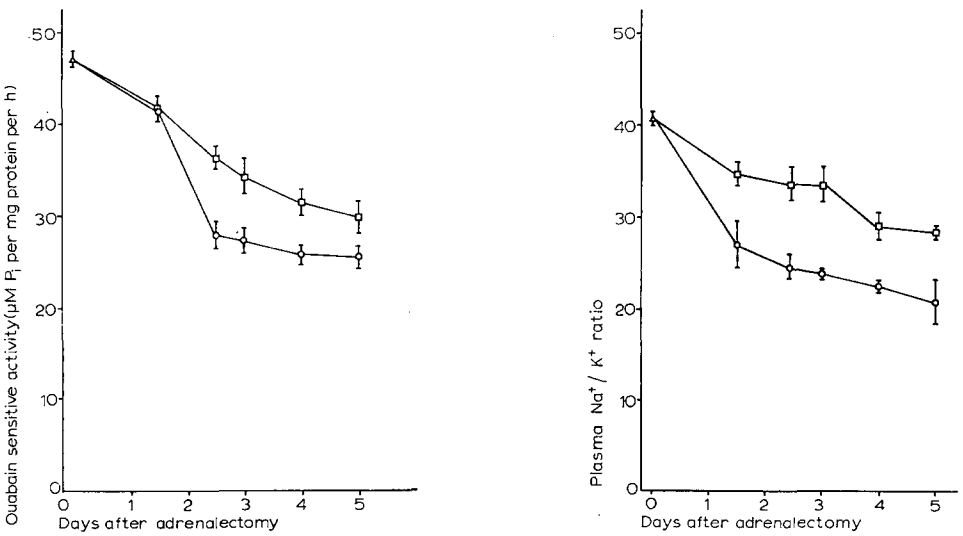

Fig. 6. Time course after adrenalectomy of the ouabain-sensitive activity in $Sed_{25\ 300}$ after incubation with deoxycholate + EDTA. Groups of adrenalectomized rats with (□) and without (○) access to 0.9% NaCl were matched with normal rats and killed at varying intervals after the operation. Experimental conditions as for Table IV, but only the ouabain-sensitive activity after incubation with deoxycholate + EDTA is given. At zero the results for 11 normal rats are shown. Each point represents the average of 3 adrenalectomized rats, except at 5 days where N = 4. Vertical lines represent ± S.E.

Fig. 7. The rate of change in the plasma Na^+/K^+ ratio calculated from the plasma Na^+ and K^+ concns. in mmoles/l plasma of the adrenalectomized rats with (□) and without (○) access to 0.9% saline used in Fig. 6. Blood samples were taken at the time of sacrifice. Number of rats as for Fig. 6. Vertical lines represent ± S.E.

adrenalectomy was enhanced from 23% in fresh to 35% in deoxycholate-treated microsomes.

In the kidneys from 6 sham-operated rats, activity of the enzymes was in the range shown for normal rats in Table IV.

Fig. 6 shows the rate of decrease in ouabain-sensitive activity in kidneys from rats killed at varying intervals after adrenalectomy. A gradual decline in ouabain-sensitive activity after incubation with deoxycholate+EDTA was observed for adrenalectomized rats supported with 0.9% NaCl solution. The level of activity observed in rats killed 7 to 9 days after the operation (Table IV) was reached after 5 days. When substitution with 0.9% NaCl was omitted and the adrenalectomized rats were kept on the usual diet and tap-water alone, the gradual decline in enzyme activity was replaced by a sudden decrease in activity between 1.5 and 2.5 days after the operation. As early as 3 days after adrenalectomy the ouabain-sensitive activity was more than 40% below the activity in kidneys from normal rats. The activities measured 2.5, 3 and 4 days after the operation were significantly lower than the values observed for saline-supported rats. In both groups the ouabain-insensitive or Mg^{2+}-activated enzyme remained at the normal level.

Fig. 7 demonstrates the well known changes in the plasma Na^+/K^+ ratio calculated from the plasma sodium and potassium concentrations of the adrenalectomized rats with and without access to saline. It is seen that the changes in the plasma Na^+/K^+ ratio developed gradually and that the per cent decrease in the plasma Na^+/K^+ ratio measured 5 days after adrenalectomy (30% and 49% below the level of normal rats) was of the same order of magnitude as the decrease in ouabain-sensitive activity of $Sed_{25\ 300}$ shown in Fig. 6 (38% and 46%).

DISCUSSION

The analysis of distribution of the $(Na^+ + K^+)$-activated enzyme in kidneys from normal and adrenalectomized rats was complicated by difficulties in measurement of total enzyme activity in the subcellular fractions. As with other enzymes bound to subcellular particles[8,18], activity of this enzyme is highly dependent on the handling of the material and therefore the conditions for activity measurement have to be properly defined.

SKOU[1] found that the presence of deoxycholate and EDTA during homogenization made it possible to prepare the $(Na^+ + K^+)$-activated enzyme system from mammalian brain and kidney with high specific activity. Recently J. C. SKOU (personal communication) demonstrated that treatment with deoxycholate + EDTA of the subcellular fractions prepared from bovine kidney in pure buffered sucrose media caused a striking increase in activity of the $(Na^+ + K^+)$-activated enzyme. CHAN[14] has described a similar effect of sodium dodecylsulfate on human erythrocyte membrane ATPase .

In this study maximum activity is defined as the highest ouabain-sensitive activity obtained by incubation with deoxycholate without inhibition of the enzyme. This can be controlled by the constancy of the Mg^{2+}-activated ATPase, which is more sensitive to deoxycholate than the $(Na^+ + K^+)$-activated enzyme.

In the fresh preparations the decrease in activity after adrenalectomy was moderate with the exception of the more than 50% reduction in activity observed in

$Sed_{105\,000}$. This fraction however contained less than 10% of the total ($Na^+ + K^+$)-activated enzyme found in rat kidney. The results for the fresh $Sed_{105\,000}$ agree with the findings of LANDON, JAZAB AND FORTE[5], who analyzed a light microsomal fraction dialyzed for 20 h in the presence of EDTA. Dialysis caused a reduction in activity with Mg^{2+} alone to about 55% of the original value and the specific activity of the ($Na^+ + K^+$)-activated enzyme seems lower than after incubation of $Sed_{105\,000}$ with deoxycholate + EDTA. The effect of deoxycholate cannot be explained by a conversion of the Mg^{2+}-activated to the ($Na^+ + K^+$)-activated enzyme caused by removal of endogenous bound cations[3,15] because the detergent can increase the activition by $Na^+ + K^+$ without considerable changes in the activity with Mg^{2+} alone.

CHIGNEL AND TITUS[6] found that adrenalectomy caused a 40 to 50% reduction in activity of the ($Na^+ + K^+$)-activated enzyme in rat kidney homogenates prepared in the presence of deoxycholate and EDTA. Differences in assay conditions and the presence of deoxycholate during homogenization can explain that the reduction is greater than that found for saline supported adrenalectomized rats in the present study.

When activity per mg tissue in whole kidney is compared, the reduction in activity of the ($Na^+ + K^+$)-activated enzyme after adrenalectomy is moderate (12%) in the fresh preparations but increases to 37% after incubation with deoxycholate + EDTA. Deoxycholate also increases the per cent difference found by assaying the microsomal fractions. These data indicate that part of the reduction in activity of the enzyme can be ascribed to a change in the action of the detergent on the preparations from adrenalectomized rat kidneys. It is not possible from the present experiments to decide whether the change in the response to deoxycholate is due to an unmasking of a true decrease in total activity of the ($Na^+ + K^+$)-activated enzyme in adrenalectomized rat kidney or to a lack of ability of the membrane material to undergo the alterations whereby the detergent changes the response to cations. A similar concentration-dependent activation by deoxycholate has been shown for enzymes bound to mitochondria (cytochrome oxidase[16] (ferrocytochrome *c*:oxygen oxidoreductase, EC 1.9.3.1); ATPase[13] (ATP phosphohydrolase, EC 3.6.1.3)) and microsomes (glucose 6-phosphatase[17] (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9); NADH cytochrome *c* reductase[18] (NADH:cytochrome *c* oxidoreductase, EC 1.6.99.3); NADH diaphorase[18] (NADH:2,6-dichlorophenolindophenol oxidoreductase, EC 1.6.99.2)). This makes a specific effect of the detergent on the ($Na^+ + K^+$)-activated enzyme less probable and indicates rather that the action of deoxycholate on the enzyme is secondary to changes in the state of the structure which are associated with this enzyme.

From these considerations it is also less probable that the effect of deoxycholate *in vitro* has any direct physiological significance. All attempts to copy the action of deoxycholate with the various adrenal steroids or to restore the activity of the enzyme from adrenalectomized rat kidney by treatment *in vitro* with adrenal steroids before and during incubation with deoxycholate + EDTA have hitherto been unsuccessfull.

The kinetic data obtained indicate a change in amount of enzyme rather than alterations in the activating effect of $Na^+ + K^+$ or in the apparent K_m for ATP and corroborate the findings of CHIGNEL AND TITUS[6]. The sensitivity to ouabain was the

same after adrenalectomy. The relation between these results and the finding of unchanged levels in microsomes of the Mg^{2+}-activated, ouabain-insensitive enzyme after adrenalectomy is uncertain, since the function of this enzyme and its possible relationship to the ($Na^+ + K^+$)-activated enzyme is partly unknown[1].

In contrast to other observations[5,6] it is shown that the supply of sodium to the adrenalectomized rats has an influence on activity of the enzyme. This finding suggests that at least part of the ($Na^+ + K^+$)-activated enzyme in rat kidney is not primarily under the control of the adrenal cortex, but is sensitive to the ionic imbalance caused by adrenalectomy. A high sodium intake can postpone and partly prevent the decrease in activity of the enzyme after adrenalectomy, but it is at present not clear whether this is due to a direct influence of sodium on activity of the enzyme. The effect can be secondary to the accompanying changes in K^+ concentration or to an action of humoral factors other than adrenal steroids. A direct influence of Na^+ on activity of the ($Na^+ + K^+$)-activated enzyme has been suggested in an attempt to explain the enhancement of metabolism by Na^+ within the epithelial cells of toad bladder, but differences in activity could not be demonstrated[19].

The decrease in ouabain-sensitive activity during developing adrenal insufficiency corresponded well to the rate of change in the plasma concentrations of sodium and potassium. This finding does not significantly relate the changes in enzyme activity to an impaired regulation of the renal excretion of sodium and potassium, since the changes in plasma concentration of these ions are also due to shifts of ions between cells and extracellular fluid[20].

A correlation between activity of the ($Na^+ + K^+$)-activated enzyme and the active transport of Na^+ and K^+ have been shown in other tissues[21,22]. To relate changes in activity of the enzyme prepared from whole kidney to the complex regulation of active transport of ions in intact kidneys is probably an oversimplification. It might however be pertinent to the present observations that micropuncture studies on adrenalectomized rat kidneys have demonstrated a considerable reduction in net transfer of sodium across the proximal tubular epithelium[9], where a major part of the filtered sodium is reabsorbed.

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