Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 181–191. Pergamon Press. Printed in Great Britain

THE ROLE OF ALDOSTERONE IN THE REGULATION OF $(Na^+ + K^+)$-ATPase IN RAT KIDNEY

PETER LETH JØRGENSEN
Institute of Physiology, University of Aarhus, Aarhus, Denmark

SUMMARY

An identity has been established between the sodium pump and an enzyme reaction, $(Na^+ + K^+)$-ATPase. The purpose of this study has been to consider the possible mechanisms of action of aldosterone from this enzymatic aspect.

A gradual decline in activity of $(Na^+ + K^+)$-ATPase in kidney is seen after adrenalectomy in rats. This decrease is partly restored by aldosterone in physiological doses. The changes are most pronounced in the outer medulla of the kidney.

Tracer studies show that the changes in activity are due to changes in the number of enzyme sites rather than to changes in the molecular activity of the enzyme.

The increase in amount of $(Na^+ + K^+)$-ATPase seen after repeated injections of aldosterone, e.g. + 48% in the outer medulla after 24 h, is interpreted as a chronic adaptation to the sustained increase in reabsorbtion of sodium.

The increase in the active reabsorbtion of sodium seen after minutes or a few hours of aldosterone stimulation seems not to involve changes in amount of $(Na^+ + K^+)$-ATPase. A review of the properties of the enzyme in kidney suggests that a control of the intracellular concentration of sodium provides a sensitive mechanism for acute adaptation, i.e. regulation of the velocity of the enzyme reaction without changing the amount of enzyme present.

INTRODUCTION

ALDOSTERONE stimulates the active transcellular transport of sodium in epithelia. Directly or indirectly, the sodium pump is therefore a target for this hormone.

Due to the work of Skou *et al.* [1], an identity has been established between the sodium pump and an enzyme reaction, the $(Na^+ + K^+)$-ATPase. It is attractive to try to consider the possible mechanisms of action of aldosterone from this enzymatic aspect.

In principle, aldosterone can control the rate of this metabolic pathway by modification of the activity of $(Na^+ + K^+)$-ATPase already present in the plasma membranes or by alteration of the amount of enzyme in the kidney tissue.

It is the purpose of this communication, first to investigate whether changes in amount of $(Na^+ + K^+)$-ATPase can play a role in the regulation by aldosterone of the excretion of cations, and secondly to consider the possibilities for modulation of the activity of $(Na^+ + K^+)$-ATPase already present in the kidney.

DETERMINATION OF THE AMOUNT OF $(Na^+ + K^+)$-ATPase IN KIDNEY

This analysis is difficult because 60–80% of the enzyme activity in homogenates and in subcellular fractions is latent. The latent enzyme activity is demasked by incubation with low concentrations of detergent [2]. The latency is apparently due to transformation of the plasma membrane into vesicles during the homogenization procedure. The enzyme requires an effect of K^+ on one side of the membrane, in the intact cell the outside, and an effect of Na^+, Mg^{2+} and ATP from the other side, the inside of the membrane. The demasking by detergents can be explained by opening of the vesicles resulting in free access of substrate and activators to their respective sites on the membrane [2]. Procedures for evaluation of the optimum conditions for activation by detergents and for quantitative analysis of the enzyme have been described [2, 3].

THE EFFECT OF ADRENALECTOMY ON THE AMOUNT OF $(Na^+ + K^+)$-ATPase IN RAT KIDNEY

A gradual decline in the activity of $(Na^+ + K^+)$-ATPase in kidney is observed after adrenalectomy in rats supported with 0·9% saline (Fig. 1), (cf. Refs. 4–7). A stable level of activity, 35–46% below the activity in normal rats, is reached after 5–6 days. When substitution with saline is omitted, a sudden decrease in activity is seen after 2 days and the activity of $(Na^+ + K^+)$-ATPase measured 3, 4 and 5 days after adrenalectomy is significantly lower than the values observed for saline-supported rats [7].

In an attempt to localize the effect of adrenalectomy, the activity of $(Na^+ + K^+)$-ATPase was measured in subdivisions of the kidney. It was found that the decrease in activity after adrenalectomy was more pronounced in the outer medulla than in the outer cortex [3, 8] (cf. Fig. 5). This suggests that there is an effect on the activity of $(Na^+ + K^+)$-ATPase in the ascending broad limbs of Henle, but does not exclude the possibility of an effect on the activity in proximal tubular segments.

To show that the observed changes in activity of $(Na^+ + K^+)$-ATPase are in

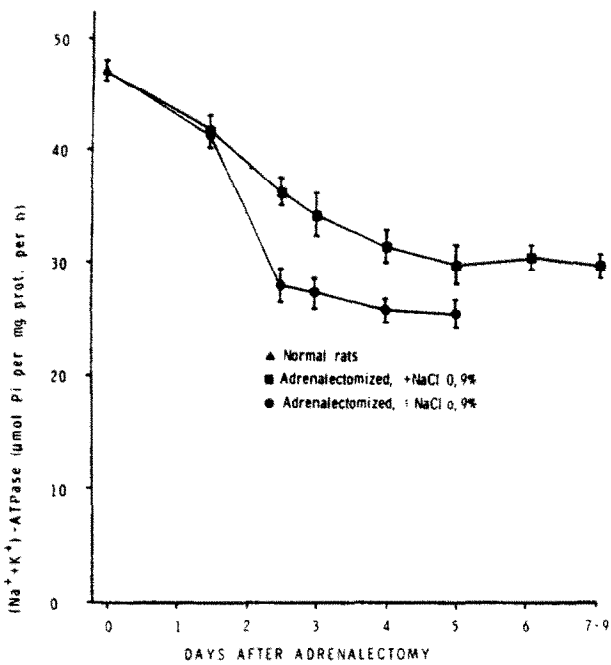

Fig. 1. The time course of the changes in activity of $(Na^+ + K^+)$-ATPase after adrenalectomy in rats with (■) and without (●) access to 0·9% NaCl. A microsomal fraction was prepared by differential centrifugation of homogenates of whole kidneys [7]. Aliquots of the microsomal fractions were incubated in 1 ml with 1·0 mg sodium deoxycholate per ml, 3 mM EDTA, 50 mM imidazole (pH 7·5, 20°C) at 0°C. After 30 min, 25 μl was transferred to test tubes (1 ml) containing 3 mM Mg^{2+}, 130 mM Na^+, 20 mM K^+, 3 mM ATP (Tris salt), 30 mM histidine (pH 7·5, 37°C). After 10 min at 37°C the reaction was stopped with 100 μl 50% TCA, and inorganic phosphate was measured. $(Na^+ + K^+)$-ATPase was calculated as the difference in activity with and without 1 mM ouabain added to the test tubes. At zero the results for 11 normal rats are shown. Each point represents the average of 3–4 adrenalectomized rats. Vertical lines represent ±S.E.

fact due to a difference in the content of enzyme protein in the tissue requires purification of the enzyme to a homogeneous state. Pure preparations of $(Na^+ + K^+)$-ATPase are at present not available, but preparations of relatively high purity can be obtained from the outer medulla of rabbit [9] and rat kidney [8].

Figure 2 shows an experiment in which a particulate fraction [3] from the outer medulla of normal and adrenalectomized rats was used as a sample in an isopycnic-zonal gradient centrifugation. In this plot [10], the area of the columns is proportional to the amount of enzyme. The particulate fraction contains all ATPase activity in homogenates of the tissue [3]. It is seen that the total area of the columns is smaller for the preparation from adrenalectomized rats than for the preparation from normal rats. A major part of the enzyme is collected in fractions with a density between 1·13 and 1·15 g/ml. In these fractions the specific activity of $(Na^+ + K^+)$-ATPase in the preparation from adrenalectomized rats is 250–350 μmoles Pi per mg protein per h, and 40–50% lower than in the preparation from normal rats, 500–650 μmoles Pi per mg protein per h.

Studies on similar preparations from the outer medulla of rabbit kidney [9] show that the high activity of $(Na^+ + K^+)$-ATPase is due to the isolation membranes with a high density of enzyme sites per unit membrane area. A further study of the relationship between these membrane fragments and the intact

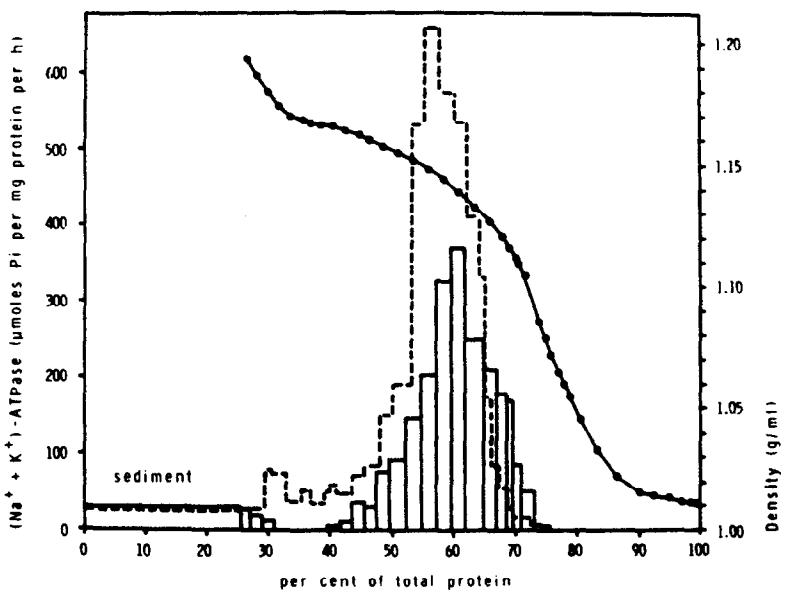

Fig. 2. The distribution of $(Na^+ + K^+)$-ATPase in fractions obtained by isopycnic-zonal gradient centrifugation [2] of particulate fractions from the outer medulla of normal (——) and of adrenalectomized rats (——). (●—●) is the density of the fractions. The kidneys from 15 normal and 15 adrenalectomized rats were dissected [3]. 1·0 g of tissue from the outer medulla of each group was used for preparation of the particulate fraction [3]. The samples for the zonal centrifugations contained 60·4 (N) and 61·3 (A) mg of protein and were prepared by suspension of the particulate fractions in 100 ml. 0·6 mg sodium deoxycholate per ml, 2 mM EDTA, 25 mM imidazole, pH 7·0. After incubation for 30 min at 20°C the samples were pumped into a Backmann Ti-14 zonal rotor and centrifuged at 40,000 rev/min for 90 min ($\omega^2 t = 10{\cdot}4 \times 10^{10}$) using a sucrose gradient ranging from 15 to 45% (w/v). Enzyme analysis as in Fig. 1. The relative protein content of the fractions is shown on the abscissa [10].

plasma membrane of the cells in the broad limb of Henle, may show whether the change in membrane area or to a change in the density of enzyme sites per unit membrane area.

To investigate if the decrease in the activity of $(Na^+ + K^+)$-ATPase after adrenalectomy is due to a decrease in the number of enzyme sites in the preparation or to a change in the molecular activity of the enzyme, an attempt was made to measure the binding of [H^3]ouabain. However, accurate measurements of ouabain binding to rat kidney preparations are impossible because the enzyme in rat kidney has a low affinity to ouabain[3, 11]. Instead, the incorporation of [^{32}P] from $AT^{32}P$[1] was used as a measure of the number of enzyme sites. This is justified because the same quantitative relationship between [^{32}P] incorporation and $(Na^+ + K^+)$-ATPase activity is found for a series of preparations with widely different specific activities[12]. Furthermore, the phosphorylation capacity is equal to the number of ATP binding sites in preparations of $(Na^+ + K^+)$-ATPase from kidney[13].

Figure 3 shows that there is a linear relationship between the $(Mg^{2+} + Na^+)$-stimulated incorporation of [^{32}P] and the specific activity of $(Na^+ + K^+)$-ATPase for preparations obtained from the outer medulla of normal and adrenalectomized rats. The molecular activity, calculated from the slope of this line, is the same for

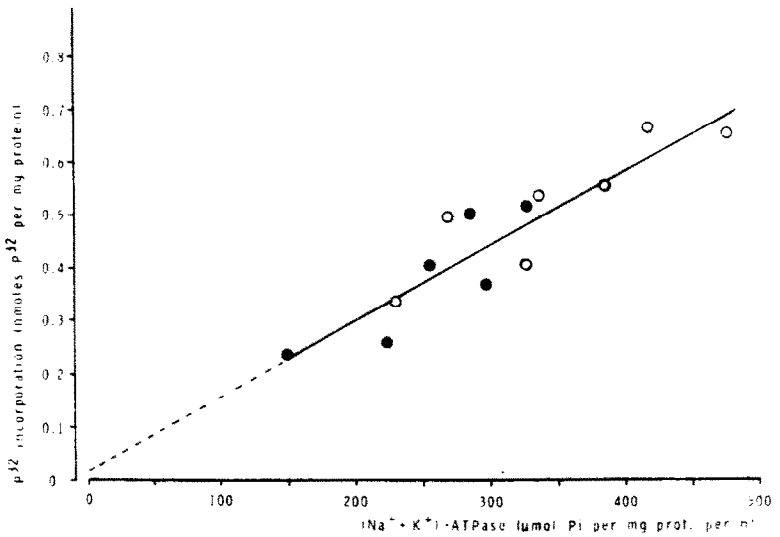

Fig. 3. The relationship between the specific activity of $(Na^+ + K^+)$-ATPase and the incorporation of [^{32}P] from $AT^{32}P$ for preparations obtained by isopycnic-zonal gradient centrifugation of particulate fractions from the outer medulla of normal (o) and adrenalectomized (•) rats. Aliquots of the preparations corresponding to 0·3–0·4 mg protein were incubated with 30 mM $AT^{32}P$, 3 mM Mg^{2+}, 100 mM Na^+ or 100 mM K^+, 30 mM Tris–HCl (pH 7·4, 37°C) in a total volume of 3 ml for 50 sec at 0°C. The reaction was stopped by addition of 3 ml. 8% perchloric acid and the protein was sedimented by centrifugation at 48,000 × *g* for 30 min. The pellet was washed 3 times with 10 mM KH_2PO_4, 0·1% PCA, 1 mM ATP and dissolved in 0·25 ml. 1 M NaOH in a water bath at 60°C for 30 min. 0·2 ml of the solution was transferred to counting vials with 10 ml of scintillator and counted in a Packard TriCarb scintillation counter. 25 μl of the solution was used for protein determination[3]. [^{32}P] incorporation was calculated as the difference in labelling after incubation with 100 mM Na^+ and with 100 mM K^+. Enzyme analysis as in Fig. 1.

the two sets of preparations, namely 11800 Pi $\times$ min^{-1}. This value is within the range previously found for $(Na^+ + K^+)$-ATPase [2. 12. 14].

The experiments in Figs. 2 and 3 thus both support the inference that the changes in activity of $(Na^+ + K^+)$-ATPase after adrenalectomy are due to changes in the amount of enzyme in the tissue.

THE EFFECT OF ALDOSTERONE ON THE AMOUNT OF $(Na^+ + K^+)$-ATPase IN KIDNEYS OF ADRENALECTOMIZED RATS

Treatment of rats, adrenalectomized 6–7 days earlier, with repeated injections of aldosterone in physiological doses increases the activity of $(Na^+ + K^+)$-ATPase in the kidney within the first 24 h of treatment (Fig. 4) [3]. After a lag period of 6 h a significant increase in activity is observed after 14 and 24 h. Prolongation of the treatment does not increase the activity much further and normal levels are not attained within 44 h. Corticosterone in doses within the range of the secretion rate of the rat adrenal glands, has no effect on the activity of $(Na^+ + K^+)$-ATPase within the first 24 h of treatment. After 48–64 h, corticosterone restores the activity to normal levels, while aldosterone at best increases the activity to a level

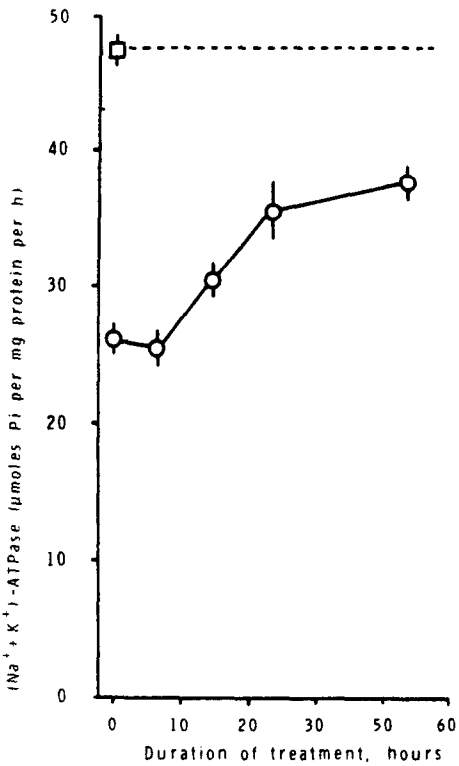

Fig. 4. The time course of the changes in activity of $(Na^+ + K^+)$-ATPase in the microsomal fraction during treatment of adrenalectomized rats with 5 μg aldosterone per 100 g rat at 4·5 h intervals [3]. The rats were adrenalectomized 7 days before the start of the treatment and had free access to both tap water and 0·9% NaCl solution. The treatment was started at the indicated time before sacrifice, and all rats were killed at the same time. Preparation of enzyme and analysis as in Fig. 1. Each point represents the average values $\pm$S.E. for 5 rats. The values of normal rats (□) are indicated by the dotted line.

midway between the activity of adrenalectomized and of normal rats. However, the pronounced differences in lag period and in dose response between the effects of aldosterone and of corticosterone make it unlikely that the effect of aldosterone is due to the known glucocorticoid effects of this hormone [3].

The time course of the change in activity of $(Na^+ + K^+)$-ATPase in preparations from the outer cortex, inner cortex, and outer medulla was measured in an attempt to localize the effect of aldosterone and to see if an increase in activity is detectable within the first few hours of treatment. It is seen from Fig. 5 that an effect of aldosterone on the enzyme activity in the preparations from the outer medulla is apparent after 6 h, but that 14 (+20%, $p < 0{\cdot}05$) and 24 h (+48%, $p < 0{\cdot}005$) of treatment is required to lead to a significant change. In the preparation from the inner cortex the increase in activity is significant after 24 h (+48%, $p < 0{\cdot}005$). Aldosterone has no significant effect on the activity of $(Na^+ + K^+)$-ATPase in the outer cortex.

During the period of treatment with aldosterone the plasma (Na^+) rose almost linearly at a rate of about 1 mM per 6 h (Fig. 5), indicating a sustained effect of the

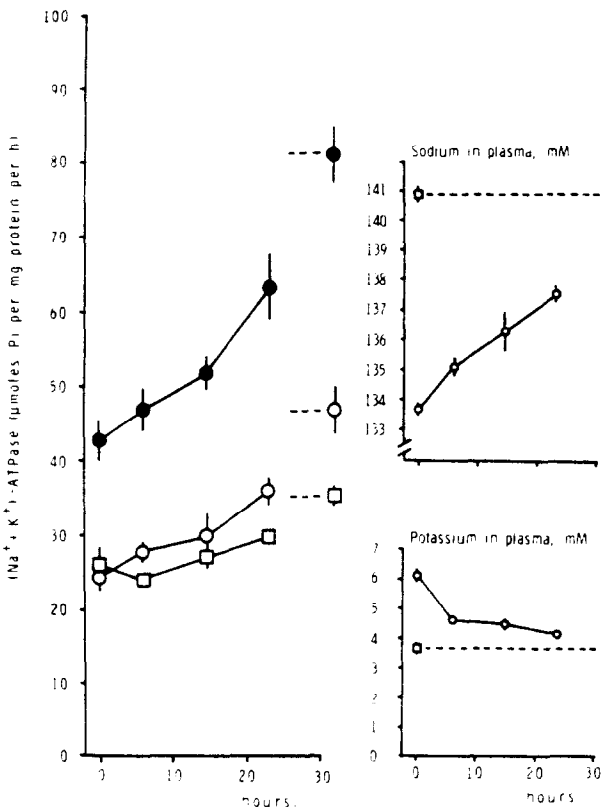

Fig. 5. The time-course of the changes in activity of $(Na^+ + K^+)$-ATPase in the outer medulla (●), inner cortex (○), outer cortex (□), and in plasma electrolytes during treatment with aldosterone, 5 μg per 100 g rat intraperitoneally at 4·5 h intervals. Treatment of each group was started at the indicated time before sacrifice, and all rats were killed at the same time. The particulate fraction was prepared and incubated with 0·6 mg sodium deoxycholate per ml., 2 mM EDTA, 50 mM imidazole, pH 7·0 (20°C). After 30 min at 20°C 25 μl. was transferred to test tubes and the enzyme activity was measured as in Fig. 1 [3].

repeated injections of aldosterone on the reabsorption of Na^+. The rate of decrease in the concentration of K^+ was highest during the first 6 h of treatment.

The increase in amount of $(Na^+ + K^+)$-ATPase is first detectable after more than 6 h of treatment with aldosterone, whereas the effect of aldosterone on the excretion of Na^+ in the urine is maximal 1–2 h after injection [15]. The data therefore indicate that the increase in the active reabsorption of sodium seen after minutes or a few hours of stimulation by aldosterone can be achieved only by a change in the activity of $(Na^+ + K^+)$-ATPase present in the tissue, since an increase in the amount of enzyme requires a longer time.

MODULATION OF THE ACTIVITY OF $(Na^+ + K^+)$-ATPase WITHOUT CHANGING THE AMOUNT OF ENZYME PRESENT

There are several possible mechanisms by which aldosterone can change the activity of enzyme present. Aldosterone can, through an effect on other systems, alter the concentrations of substrate or activators in the environment of the enzyme. The hormone can act directly on the enzyme to change its affinity to substrate or activators or it can remove inhibitors or repressor molecules to transform inactive enzyme molecules into active sites.

It has been proposed that the primary effect of aldosterone is to facilitate the entry of Na^+ into the epithelial cells [16, 17] or to increase the rate of ATP synthesis [18]. To investigate how these changes may alter the enzyme activity, the effect of Na^+, K^+, and ATP on the activity of $(Na^+ + K^+)$-ATPase from the outer medulla of rat kidney has been reexamined using a range of concentrations presumably found in kidney tissue.

Figure 6 shows the effect of $Na^+ + K^+$ on the activity of the enzyme. In order to simulate the conditions of the intact cell isotonicity was maintained by exchanging Na^+ for K^+ [19]. $(Na^+ + K^+)$-ATPase behaves as if it has two sites with affinities for Na^+ and K^+ [20, 21]. One site, the K-site, is in contact with the extracellular phase. At this site the K/Na ratio for half maximum activation in Fig. 6

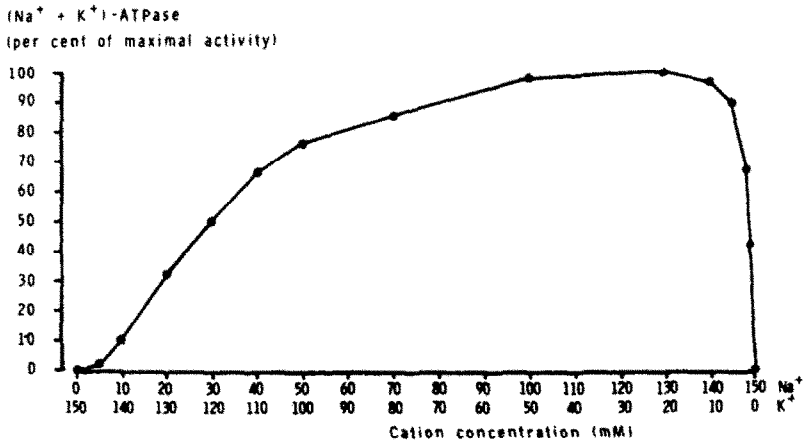

Fig. 6. The activation by $Na^+ + K^+$ of the ATP bydrolysis by a microsomal fraction prepared from the outer medulla of normal rats. The ratio between Na^+ and K^+ in the test tubes was varied at a constant ionic strength. ATP 3 mM, Mg^{2+} 3 mM. Incubated at 37°C and pH 7·5 for 5 min. The results are expressed in per cent of the activity with Na^+ 130 mM, K^+ 20 mM (260 μmol Pi per mg protein per h).

is 1·1/148·9, i.e. an apparent affinity for K^+ more than 100 times higher than for Na^+. This site must be nearly saturated with K^+ at the concentrations of Na^+ and K^+ in the extracellular fluid. The other site, the Na-site, faces the intracellular water phase. The Na/K ratio for half maximum activity in Fig. 6 is 30/120, i.e. an apparent affinity for Na^+ about 4 times higher than for K^+. In the presence of intracellular concentrations of K^+ (130–140 mM) and Na^+ (10–20 mM) the saturation of this site with Na^+ is low (10–30%) and increases steeply when the concentration of sodium is increased. It is reasonable to assume that the velocity of the enzyme reaction is proportional to the fractional saturation of the enzyme with Na^+ at the Na-site and K^+ at the K-site. A control of the intracellular concentration of Na^+ therefore provides a sensitive and rapid mechanism for regulation of the velocity of this enzyme reaction (cf. Refs. 20, 21).

In Fig. 7 the relationship between the ATP concentration and the enzyme activity is shown. The concentration of ATP necessary for half maximum activity of $(Na^+ + K^+)$-ATPase at the different concentrations of Na^+ and K^+ used is lower than 0·15 mM. Virtually no change in activity is observed when the ATP concentration is increased from 0·75 to 2·0 mM.

Measurements at low ATP concentrations suggest that the apparent K_m for the hydrolysis of ATP may be much lower than 0·15 mM, namely 0·2 uM [22] and 3·3 uM [23]. Furthermore, studies of the binding of ATP to preparations of $(Na^+ + K^+)$-ATPase from brain have shown that the dissociation constant for enzyme-ATP is 0·1–0·7 uM, depending on the K^+ concentration [24]. The same values have been found for preparations from kidney [13].

In rat kidney, the concentration of ATP is about 2 μmoles per g wet tissue [25, 26], suggesting that the concentration of ATP in the intracellular water phase is above 2 mM. This is much higher than the concentration of ATP necessary for

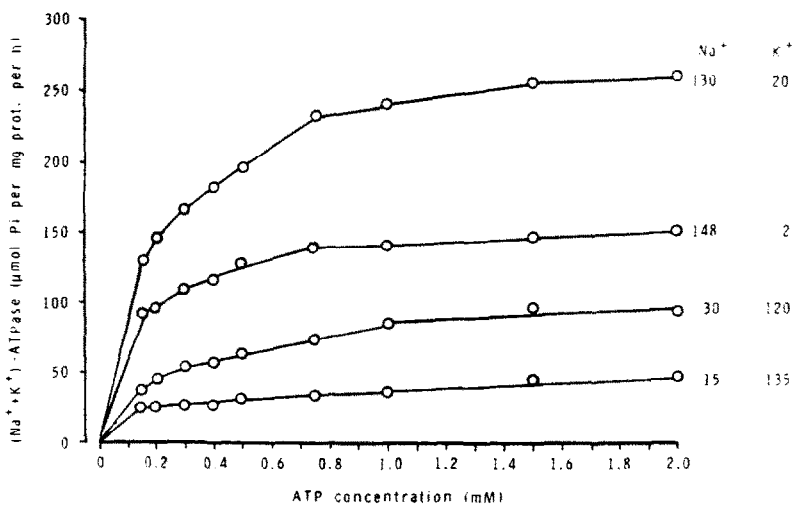

Fig. 7. The relationship between the activity of $(Na^+ + K^+)$-ATPase and the concentration of ATP for a microsomal fraction prepared from the outer medulla of normal rats. The ratio between ATP and Mg^{2+} in the test tubes was 1 : 1. $(Na^+ + K^+)$-ATPase activity was calculated as the activity measured in the presence of $Na^+ + K^+$ as indicated in the figure minus the activity in the presence of 150 mM K^+ alone. Each point represents the average value of the activities measured after 1, 3 and 5 min of incubation at 37°C.

saturation of the enzyme. It seems therefore not likely that alterations in the concentration of ATP play a role in the regulation of $(Na^+ + K^+)$-ATPase.

Aldosterone has no influence *in vitro* on the velocity of the enzyme reaction and it seems that the hormone is without influence on the apparent affinity constants of $(Na^+ + K^+)$-ATPase [3, 5]. The possibilities that aldosterone may act to relieve inhibition of the enzyme or to transform a proenzyme into an active enzyme have not been studied in detail.

ACUTE AND CHRONIC ADAPTATION OF $(Na^+ + K^+)$-ATPase

The scheme in Fig. 8 illustrates how aldosterone may regulate $(Na^+ + K^+)$-ATPase in the outer medulla of the kidneys of adrenalectomized rats. It is assumed that the increase in the active transcellular transport within minutes of a few hours of aldosterone stimulation, an *acute adaptation* [27], is secondary to an increase in the intracellular concentration of Na^+. In the example shown, an increase in the Na^+ concentration from 14 to 23 mM increases the saturation of the Na-site with Na^+ from 20 to 40% and thus leads to a 100% increase in the velocity of the enzyme reaction. In the distal tubular cells $(Na^+ + K^+)$-ATPase probably represents a major component of energy utilization. The increase in enzyme activity therefore enhances oxygen uptake and the rate of ATP synthesis.

The increase in amount of $(Na^+ + K^+)$-ATPase seen after repeated injections of aldosterone, e.g. +48% in the outer medulla after 24 h (Fig. 5), is interpreted as a *chronic adaptation* [27] to the sustained increase in the functional demands on the enzyme [3]. In the example in Fig. 8 the absolute number of sites saturated with Na^+ are kept constant. This means that the fractional saturation with Na^+ at the Na-site decreases and that the higher rate of enzyme activity can be maintained at a lower intracellular Na^+ concentration.

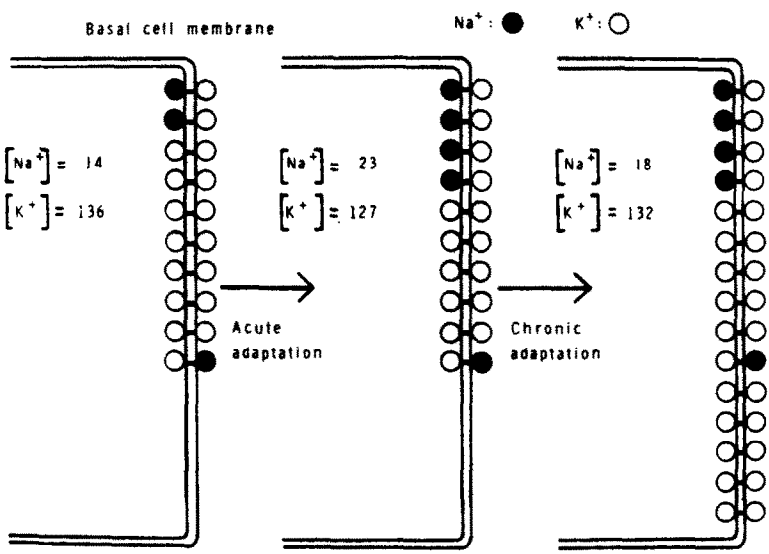

Fig. 8. Acute and chronic acaptation of $(Na^+ + K^+)$-ATPase in the basal cell membrane of the tubular cells in the outer medulla of rat kidney. The concentrations of Na^+ and K^+ in the intracellular water phase are shown. (—) denotes the enzyme system, (●) sodium ions, and (○) potassium ions.

The mechanism by which the amount of $(Na^+ + K^+)$-ATPase is controlled is not known. It is tempting to forward the hypothesis that the intracellular Na/K ratio can control the rate of biosynthesis or degradation of the enzyme protein. There is at present no evidence in support of this. However, a pronounced influence of changes in the concentrations of Na^+ and K^+ on the rate of protein synthesis has been demonstrated in other systems[28].

REFERENCES

1. Skou J. C.: *Physiol. Rev.* **45** (1965) 596.
2. Jørgensen P. L. and Skou J. C.: *Biochim. biophys. Acta* **233** (1971) 366.
3. Jørgensen P. L.: *Biochim. biophys. Acta* **192** (1969) 326.
4. Landon E. J., Jazab N. and Forte L.: *Am. J. Physiol.* **211** (1966) 1050.
5. Chignel C. F. and Titus E.: *J. Biol. Chem.* **241** (1966) 5083.
6. Katz A. I. and Epstein F. H.: *J. Clin. Invest.* **46** (1967) 1999.
7. Jørgensen P. L.: *Biochim. biophys. Acta* **151** (1968) 212.
8. Jørgensen P. L.: *Acta physiol. Scand.* **74** (1968) 11A.
9. Jørgensen P. L., Skou J. C. and Solomonson L. S.: *Biochim. biophys. Acta* **233** (1971) 381.
10. De Duve C., Berthet J. and Beaufay H.: *Progr. Biophys.*, **9** (1959) 325.
11. Ahmed K. and Judah J. D.: *Biochim. biophys. Acta* **93** (1964) 603.
12. Bader H., Post R. L. and Bond G.: *Biochim. biophys. Acta* **150** (1968) 41.
13. Hegyvary C. and Post R. L.: *J. Biol. Chem.* **246** (1971) 5234.
14. Baker P. F. and Willis J. S.: *Biochim. biophys. Acta* **183** (1969) 646.
15. Fimognari G. M., Fanestil D. D. and Edelman J. S.: *Am. J. Physiol.* **213** (1967) 954.
16. Crabbe J., *Nature* **200** (1963) 787.
17. Sharp G. W. G. and Leaf A.: *Physiol. Rev.* **46** (1966) 593.
18. Edelman I. S.: *Functions of the Adrenal Cortex*, Vol. I (Edited by K. W. McKerns). North Holland, Amsterdam (1968) p. 79.
19. Skou J. C.: *Biochim. biophys. Acta* **58** (1962) 314.
20. Skou J. C.: *Biochim. Biophys. Acta* **23** (1957) 394.
21. Skou J. C.: *Biochim. biophys. Acta* **42** (1960) 6.
22. Neufeld A. H. and Levy H. M.: *J. Biol. Chem.* **244** (1969) 6493.
23. Saito M.: *Ann. Rept. Biol. Works* (Faculty of Science, Osaka University, Japan) **17** (1969) 15.
24. Nørby J. G. and Jensen J.: *Biochim. biophys. Acta* **233** (1971) 104.
25. Gerlach E., Bader W. and Schwoerer W.: *Arch. Ges. Physiol.* **272** (1961) 407.
26. Jones N. F. and Welt L. G.: *Am. J. Physiol.* **212** (1967) 939.
27. Ashmore J. and Weber G.: *Carbohydrate Metabolism and it's Disorders*, Vol. I (Edited by F. Dickens, P. J. Randle and W. J. Whelan). Academic Press, London (1968) p. 335.
28. Appel S. H., Autilio L., Festoff B. W. and Escueta A. V.: *J. Biol. Chem.* **244** (1969) 3166.

DISCUSSION

Kirsten: How did you treat your animals in the first 24 h: what were your intervals of injections of aldosterone? And my second question is: how do your results compare with those of Landon? He claims that there is no effect on the sodium/potassium dependent ATPase by aldosterone.

Jørgensen: The rats had access to both tap water and to saline. The dose of aldosterone was 5 micrograms per 100 g rat intraperitoneally every 4½ h.

In reference to Landon's results: Landon (*Am. J. Physiol.* **211** (1966) 1050) studied the effect of aldosterone in two ways. First he found that daily injections of aldosterone for 5 days could hinder the fall in activity of $(Na^+ + K^+)$-ATPase adrenalectomy. Secondly, he found no change in the activity of $(Na^+ + K^k)$-ATPase after treatment of adrenalectomized rats with aldosterone for 3 h. This is in agreement with our results, since we found an increase in activity of $(Na^+ + K^+)$-ATPase after about 14 h of treatment with aldosterone.

Wiederholt: What was the method of your preparation?

Jørgensen: The particulate fraction is obtained by sedimentation of all particles in the homogenate at high speed in the ultracentrifuge. The soluble phase or cell sap is devoid of ATPase activity and contains 40% of the protein in the homogenate. All ATPase activity is found in the particulate fraction and the specific activity of $(Na^+ + K^+)$-ATPase is relatively high due to the removal of inactive protein.

Wiederholt: What are your thoughts about the method Dr. Ebel used?

Jørgensen: In the preparation obtained by isopycnic-zonal centrifugation of the particulate fraction from the outer medulla of normal rats the specific activity of $(Na^+ + K^+)$-ATPase is 500–650 μmoles Pi per mg protein per hour. In this preparation the enzyme activity is bound to structures with the morphological and chemical characteristics of plasma membranes. In the preparations of plasma membranes from kidney used by Ebel and coworkers (H. Ebel *et al.*, *Pflügers Archiv* **324** (1971) 1) the specific activity of $(Na^+ + K^+)$-ATPase was 10–20 μmoles Pi per mg protein per h. They found an increase in the activity of both $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase after adrenalectomy and a decrease in these activities after treatment with aldosterone. We found a decrease in the activity of $(Na^+ + K^+)$-ATPase after adrenalectomy and a partial restoration of the activity after treatment with aldosterone, whereas the Mg^{2+}-ATPase activity remained constant. I suggest that the discrepancy between these results may be explained by differences in the properties of the preparations used.

De Weer: Dr. Jørgensen, you did not mention that your preparation has the highest specific activity in the world for ATPase. This might explain the discrepancy.