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THE K^+-DEPENDENT PHOSPHATASE OF RAT KIDNEY

ITS PROPERTIES AND THE EFFECTS OF MANEUVERS THAT MODIFY ($Na^+ + K^+$)-ATPase ACTIVITY

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Summary

The properties of rat renal *p*-nitrophenylphosphatase have been investigated and the effects of certain experimental maneuvers (known to modify renal ($Na^+ + K^+$)-ATPase) on *p*-nitrophenylphosphatase specific activity have been examined. The rat kidney enzyme had an optimal pH in the range 7—8 and the temperature dependence of enzyme activity (Arrhenius plot) yielded a single slope with an activation energy of 40 kJ/mol. ($Na^+ + K^+$)-ATPase assayed in the same enzyme preparation yielded a biphasic plot with activation energies of 81 and 160 kJ/mol and phase transition at 20°C. The K_m value for *p*-nitrophenyl phosphate was 1.62 mM and the $K_{1/2}$ value for potassium was 2.2—3.3 mM. ATP had a dual inhibitory effect on the potassium activation of the phosphatase: at low concentrations a non-competitive pattern was observed (K_i = 0.015 mM), and at high concentrations a typical competitive inhibitory pattern occurred (K_i = 0.04 mM). Sodium at low concentrations produced a substantial decrease in the affinity of the phosphatase for potassium (1.8 to 6 mM); higher concentrations of sodium did not have additional effects on the potassium affinity but markedly reduced the *V* value of the reaction. Ouabain (1—5 mM) inhibited basal enzyme activity by 80%. Preincubation of the enzyme with ouabain produced mild stimulation at low concentrations of the glycoside (1—100 μM) and resulted in 100% inhibition of basal activity at high concentrations (1—5 mM). Ouabain inhibited *p*-nitrophenyl phosphate activation of the phosphatase by a pattern similar to that observed with sodium: a decrease in the apparent affinity for *p*-nitrophenyl phosphate (1.62 to 3 mM) at low concentrations of the glycoside and a decrease in *V* at higher concentrations with-

out additional effects on the affinity. Ouabain inhibition of the potassium activation of the phosphatase displayed kinetics of uncompetitive inhibition. Unlike the enzyme from red blood cells, calcium, in the presence of ATP, did not result in loss of ouabain sensitivity. The specific activities of both the potassium-activated and ouabain-sensitive phosphatases changed in parallel with changes in $(Na^+ + K^+)$-ATPase under the following experimental conditions: adrenalectomy, chronic dexamethasone treatment, potassium loading, acute dexamethasone treatment and reduction of renal mass. The changes in the specific activities of the two enzymes were quantitatively correlated in the cortex and medulla following adrenalectomy and chronic dexamethasone treatment. A similar correlation was found in the cortex (but not in the medulla) after potassium loading. Stimulation by acute dexamethasone treatment of *p*-nitrophenylphosphatase in the renal cortex (but not in the medulla) was substantially larger than the corresponding stimulation of $(Na^+ + K^+)$-ATPase. After reduction of renal mass, stimulation of $(Na^+ + K^+)$-ATPase was not consistently observed. When it occurred, it was confined to the renal medulla and it was accompanied by increases in the specific activity of *p*-nitrophenylphosphatase. But the magnitude of *p*-nitrophenylphosphatase stimulation was substantially lower than that of $(Na^+ + K^+)$-ATPase. These observations and the quantitative aspects of the correlations between renal $(Na^+ + K^+)$-ATPase and *p*-nitrophenylphosphatase suggest that under all the experimental conditions examined, $(Na^+ + K^+)$-ATPase activation may reflect an increase in the number of enzyme units. In addition, the results suggest that in certain situations (acute dexamethasone treatment, reduction of renal mass), additional effects on the kinetic properties of the transport enzyme may also occur.

Introduction

In 1962, Judah et al. [1] described the presence in red cell membranes of an enzymatic activity capable of hydrolyzing *p*-nitrophenyl phosphate at neutral pH. The hydrolytic activity was activated by Mg^{2+} and K^+ and the K^+-dependent activation was completely abolished by ouabain [1]. Subsequent investigations disclosed the presence of similar activities in a variety of tissues: brain [2], kidney [2,3], gastric mucosa [4], intestinal epithelia [5], liver [2] and smooth muscle [2]. Furthermore, several properties of the K^+-dependent phosphatase including activation by cations [3], inhibition by cardiac glycosides [6], copurification with $(Na^+ + K^+)$-ATPase [7], and the presence of a phosphorylated intermediate [8,9] have provided convincing evidence in support of the concept that the K^+-activated phosphatase and $(Na^+ + K^+)$-ATPase are components of the same enzymatic unit and that the phosphatase activity corresponds to the K^+-activated site for dephosphorylation of the high-energy intermediate of the $(Na^+ + K^+)$-ATPase reaction (for a review, see Ref. 10). It should be stressed, however, that biochemical identity between the K^+-dependent phosphatase and the $(Na^+ + K^+)$-ATPase has not been unequivocally established, and studies with enzyme of brain microsomes have disclosed some remarkable differences in the inhibitory effects of ouabain, oligomycin and *N*-ethylmaleimide [9,11], and in the lipid dependence [11,12], thus suggesting that the

enzymatic activities may represent different entities [9,11,12].

The mammalian kidney possesses a high specific activity of $(Na^+ + K^+)$-ATPase [13,14]. It is generally agreed that the relatively high activity of this transport enzyme is in keeping with the high rates of transtubular reabsorption of ions (Na^+, K^+) and water [14]. A variety of experimental maneuvers (e.g., reduction of renal mass, increases in the dietary content of K^+, high protein feeding, acute and chronic administration of synthetic glucocorticoid hormones) stimulate the specific activity of renal $(Na^+ + K^+)$-ATPase [14—16]. In all of these experimental situations, the increase in the specific activity of $(Na^+ + K^+)$-ATPase appears to correlate, to some extent, with changes in the rates of transtubular ionic fluxes and accordingly, the changes in $(Na^+ + K^+)$-ATPase activity have been regarded as an adaptive mechanism of profound physiological relevance [14,16]. The mechanisms responsible for these changes in $(Na^+ + K^+)$-ATPase specific activity have not been defined. In addition, enzyme activation does not always correlate with changes in ionic fluxes, since it has been shown that the two processes can be dissociated experimentally after chronic administration of glucocorticoid hormones [16], thus suggesting that enzyme activation under certain conditions may serve purposes other than the observed changes in ion transport. Investigation of the behavior of the K^+-activated phosphatase in these experimental situations may provide insight into the activation mechanism and help in the understanding of the ultimate physiological relevance of the adaptive increase in $(Na^+ + K^+)$-ATPase specific activity. In addition, since the phosphatase activity is widely regarded as a reflection of the second step (K^+ activation) of the $(Na^+ + K^+)$-ATPase enzymatic reaction [6,3], these studies may uncover effects of these maneuvers on the partial steps of the $(Na^+ + K^+)$-ATPase reaction. Some properties of the K^+-activated phosphatase from guinea-pig kidney [3], rabbit kidney [12,17, 18], and human kidney [19] have been reported, but the properties of the rat kidney enzyme have not been investigated. Most of these studies have addressed the issue of whether the two enzymatic activities represent the same biochemical entity. In this paper, we have examined some kinetic properties of the K^+-activated phosphatase in the cortex and the medulla of the rat kidney, stressing the interaction of K^+ with other ions and substrates, and in addition, we have investigated the effect of different experimental maneuvers (that affect renal $(Na^+ + K^+)$-ATPase) on the specific activity of the K^+-dependent phosphatase.

Materials and Methods

Experiments were performed on male Sprague-Dawley rats, ranging in weight from 150 to 300 g. In the experiments on the effects of acute and chronic administration of dexamethasone female animals were used. The rats were fed a standard rat pellet diet (Purina Rat Chow No. 5012) containing 41 mequiv./g of Na^+, 0.28 mequiv./g of K^+ and 22.8% protein. Potassium loading was accomplished by substituting the drinking water by a 100 mM solution of KCl for 1 week. This maneuver resulted in a 3-fold increase in the daily load of potassium handled by the experimental animals (0.83 ± 0.23 mequiv./day per 100 g body wt. in control rats as against 2.36 ± 0.37 mequiv./day per 100 g body wt. in

K^+-loaded animals). Adrenalectomy and left unilateral nephrectomy were performed by a sub-costal approach under chloral hydrate anesthesia (36 mg/100 g body wt., intraperitoneally). Adrenalectomized animals were maintained by substituting the drinking water by 0.9% saline solution. All animals were allowed to recover from surgery for at least 5 days prior to any experimental maneuver. Dexamethasone was administered as a single dose (1—5 mg/100 g body wt., intraperitoneally) or daily for 5 days. Sham-operated control animals received identical volumes of steroid vehicle (0.15% $Na_2S_2O_3$, 1% benzyl alcohol N.F.). The animals were killed 2 h after the single or last injection of hormone by exanguination from the abdominal aorta. Unilaterally nephrectomized rats were killed 2 weeks after surgery and adrenalectomized animals 5 days to 2 weeks after surgery.

Tissue preparation

The kidneys were perfused with ice-cold 0.9% saline solution and decapsulated. The cortex and red (outer) medulla were dissected, minced and homogenized in 250 mM sucrose containing 5 mM EDTA, 0.01 mM Cleland's reagent and 5 mM histidine, pH 7.5. In some experiments, 0.1% deoxycholate was added (fresh) to the homogenizing solution. Addition of the detergent resulted in a substantial increase (2—3-fold) of the specific activity of both enzymes but did not modify the overall results. Homogenization was carried out in a glass homogenizer with a motor-driven Teflon pestle by ten strokes at top speed. The homogenate was centrifuged at $1000 \times g$ for 10 min, the supernatant of this centrifugation was diluted with homogenizing solution (1 : 4) and centrifuged at $100\,000 \times g$ for 1 h. The pellet of this centrifugation was washed in 10 mM Tris-HCl, pH 7.4., by centrifugation at $100\,000 \times g$ for 30 min and the pellet was resuspended and stored in 10 mM Tris-HCl, pH 7.5. For the experiments on nephrectomized animals, 'loose layer' fractions were obtained by differential centrifugation essentially as described by Lo et al. [20]. Enzyme specific activities were comparable with either type of preparation and remained stable for periods as long as 90 days when stored at −20°C. Repeated freeze-thawing was without effect on enzyme activity.

Enzyme assays

p-Nitrophenylphosphatase. The Mg^{2+}-dependent *p*-nitrophenylphosphatase activity was determined by incubating an appropriate volume of enzyme suspension in a reaction mixture of the following composition: 10 mM Tris-HCl, pH 7.7, 5 mM $MgCl_2$ and 5 mM *p*-nitrophenyl phosphate in a total volume of 1 ml. Preliminary experiments showed that the concentration of Mg^{2+} required for optimal activation was about 1 mM and maximal activities were obtained in the range 2—5 mM. The K^+-dependent *p*-nitrophenylphosphatase activity was defined as the increase in *p*-nitrophenyl phosphate hydrolysis observed upon addition of 1—10 mM KCl to the reaction mixture, and the ouabain-sensitive *p*-nitrophenylphosphatase activity as the inhibition of the K^+-activation of *p*-nitrophenyl phosphate hydrolysis by 0.1—1 mM ouabain. The mixtures were incubated at 37°C for 15 min. The reaction was stopped by cooling the tubes at 0—4°C and adding 0.25 ml of 1 M NaOH. *p*-Nitrophenol release was quantitated from the absorbance at 410 nM in a Zeiss spectrophotometer by using a

molar extinction coefficient for *p*-nitrophenol of $18 \cdot 10^6$ [15].

$(Na^+ + K^+)$-ATPase. Total ATPase activity was determined by incubating an appropriate volume of enzyme suspension in a reaction mixture of the following composition: 100 mM NaCl, 20 mM KCl, 3 mM ATP (Tris salt), 0.02—0.05 μCi [γ-^{32}P]ATP (spec. act. ≅3000 Ci/mM) and 50 mM Tris-HCl, pH 7.5, in a final volume of 0.1 ml. Mg^{2+}-ATPase was estimated in tandem tubes incubated in the absence of KCl or in the presence of 1 mM ouabain. The results obtained were comparable with either approach. Incubations were carried out at 37°C for 15 min; the reaction was terminated by cooling the mixtures (0—4°C) and adding 0.5 ml of a solution of 190 mM $HClO_4$ containing 0.25 mM KH_2PO_4. The P_i released was then precipitated as previously described [15] and the ^{32}P radioactivity was quantitated by scintillation counting. Protein concentrations in the enzyme preparations were determined by using the method of Lowry et al. [21].

Materials

ATP (Tris salt, vanadium-free) from equine muscle, *p*-nitrophenyl phosphate (dicyclohexyl ammonium salt), ouabain octahydrate and deoxycholic acid (sodium-salt) were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. [γ-^{32}P]ATP was obtained from New England Nuclear Corp. (Boston, MA). The purity of each batch of isotope was regularly tested by precipitation with $(NH_4)_2MoO_4$ [15]. All of the conventional chemicals were purchased from different suppliers without apparent effects on the results obtained.

Results

Effects of protein concentration, incubation time, pH, temperature and the concentrations of p-nitrophenyl phosphate and K^+ on hydrolysis of p-nitrophenyl phosphate. Hydrolysis of *p*-nitrophenyl phosphate was linear for enzyme protein concentrations in the range 2—20 μg and for incubation times from 5 to 30 min (Fig. 1). The pH profile of the renal *p*-nitrophenylphosphatase is depicted in Fig. 2. Both the K^+-activated and ouabain-sensitive activities were markedly enhanced in the pH range 7—8; maximal activity was attained at pH 7.8. In contrast, the activity of the Mg^{2+}-activated *p*-nitrophenylphosphatase was minimal at pH 7.8 and increased for pH values above or below this value. The temperature dependence of the K^+-activated *p*-nitrophenylphosphatase was investigated and compared to that of $(Na^+ + K^+)$-ATPase in the same enzyme preparation. As shown in Fig. 3, Arrhenius plots of $(Na^+ + K^+)$-ATPase produced the well known biphasic pattern consistent with the interpretation that the activation energy of the $(Na^+ + K^+)$-ATPase reaction is influenced by the physical state of the phospholipids associated with the enzyme protein [12,17]. Phase transition occurred at 20°C; the activation energy below the phase transition temperature was 161 kJ/mol and the activation energy above the phase transition temperature was 81 kJ/mol. In contrast, Arrhenius plots of the K^+-dependent *p*-nitrophenylphosphatase produced a single straight line with an activation energy of 40 kJ/mol, which is lower than the activation of the $(Na^+ + K^+)$-ATPase reaction both above and below the

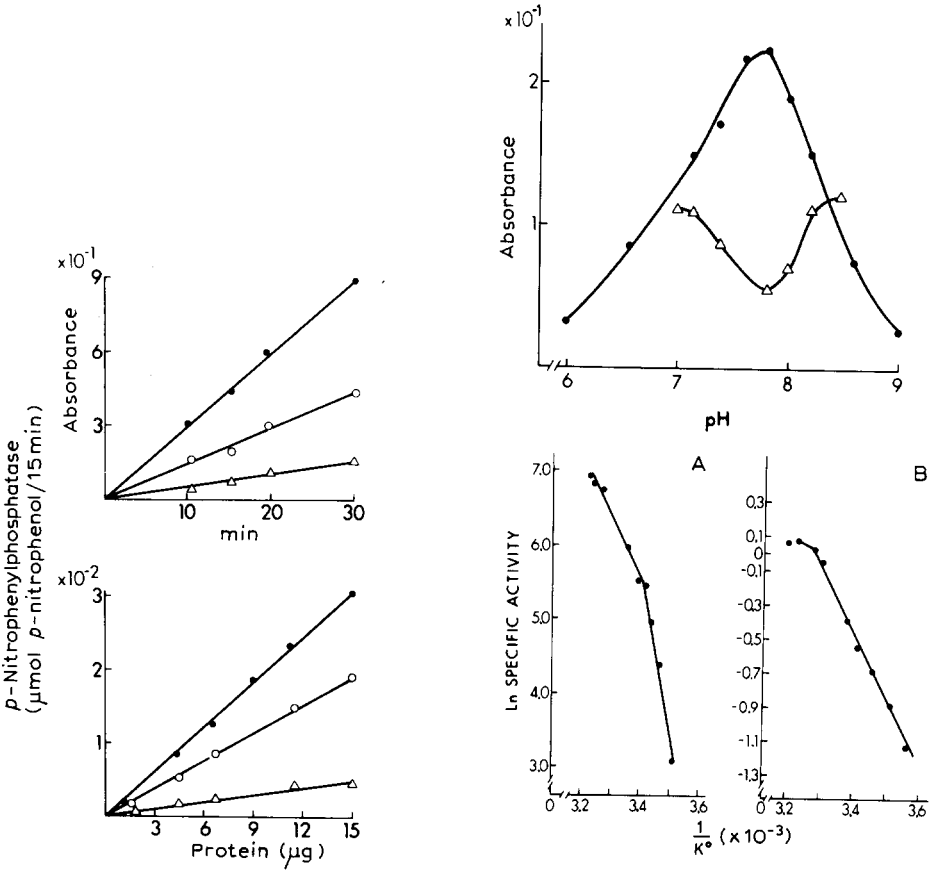


Fig. 1. (Left) Effects of incubation time and protein concentration on the activities of *p*-nitrophenylphosphatase from renal cortex. •, K^+-activated; ○, ouabain-sensitive; △, Mg^{2+}-activated.

Fig. 2. (Top right) pH dependence of the activity of Mg^{2+}-activated (△) and K^+-activated (○) *p*-nitrophenylphosphatase from rat kidney cortex.

Fig. 3. (Bottom right) Temperature dependence of the specific activities of $(Na^+ + K^+)$-ATPase (A) and K^+-activated *p*-nitrophenylphosphatase (B) from renal cortex analyzed by Arrhenius plots.

phase transition temperature. In preparations from rabbit kidney, a tissue with ouabain sensitivity markedly different to that of rat kidney [22], higher values for activation energy of *p*-nitrophenylphosphatase (46—63 kJ/mol) have been reported [17]. Differences in the methods of enzyme isolation may also explain these discrepancies. These observations clearly suggest that the K^+-dependent phosphatase is independent of the physical state of membrane phospholipids and agrees well with the concept that the K^+-dependent phosphatase corresponds to an externally located catalytic site of the $(Na^+ + K^+)$-ATPase [10,23]. It has long been recognized that the specific activity of $(Na^+ + K^+)$-ATPase in the red (outer) medulla of the kidney is substantially higher than in the cortex [24]. We examined the specific activity of *p*-nitrophenylphosphatase and $(Na^+ + K^+)$-ATPase in cortex and medulla obtained from the same set of kidneys. As shown in Table I, the specific activities of both $(Na^+ + K^+)$-ATPase

TABLE I

SPECIFIC ACTIVITIES OF $(Na^+ + K^+)$-ATPase AND *p*-NITROPHENYLPHOSPHATASE IN CRUDE HOMOGENATES AND MICROSOMAL FRACTIONS OF RENAL CORTEX AND RENAL MEDULLA

The values are the means ± S.E. of five normal rats. Specific activities of $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase are in μmol P_i/mg protein per h and of K^+- and Mg^{2+}-activated *p*-nitrophenylphosphatase in μmol *p*-nitrophenol/mg protein per 15 min. The ratios of cortical to medullary enzyme specific activities were virtually the same for $(Na^+ + K^+)$-ATPase and *p*-nitrophenylphosphatase in either crude homogenates or microsomal fractions. Likewise, the enrichment of specific activities in microsomal fractions (approx. 8-fold) was essentially similar for both enzymatic activities.

	Crude homogenate	Microsomal fraction	Microsomal fraction: crude homogenate
$(Na^+ + K^+)$-ATPase			
Cortex	1.6 ± 0.1	13.5 ± 0.7	8.7 ± 1.2
Medulla	3.7 ± 0.2	32.1 ± 2.1	8.6 ± 0.9
Medulla:cortex	2.3 ± 0.3	2.4 ± 0.3	—
Mg^{2+}-ATPase			
Cortex	7.8 ± 0.3	10.1 ± 1.0	1.3 ± 0.1
Medulla	7.3 ± 0.6	9.9 ± 0.4	1.4 ± 0.2
Medulla:cortex	0.9 ± 0.1	1.0 ± 0.1	—
K^+-activated *p*-nitrophenylphosphatase			
Cortex	0.35 ± 0.05	2.78 ± 0.27	7.9 ± 0.8
Medulla	0.81 ± 0.07	6.56 ± 1.46	8.2 ± 0.6
Medulla:cortex	2.3 ± 0.4	2.3 ± 0.3	—
Mg^{2+}-activated *p*-nitrophenylphosphatase			
Cortex	1.23 ± 0.16	1.12 ± 0.06	0.9 ± 0.1
Medulla	1.31 ± 0.18	1.29 ± 0.20	1.0 ± 0.2
Medulla:cortex	1.0 ± 0.2	1.15 ± 0.09	—

and K^+-activated *p*-nitrophenylphosphatase were substantially higher in medulla than in cortex and the ratios of medullary to cortical enzyme specific activities were virtually equal for $(Na^+ + K^+)$-ATPase and K^+-activated *p*-nitrophenylphosphatase both in crude homogenates and microsomal fractions. In contrast, the specific activities of Mg^{2+}-ATPase and Mg^{2+}-activated *p*-nitrophenylphosphatase were essentially the same in medulla and cortex and the corresponding ratios were not different from unity. Activation of the K^+-dependent phosphatase by different concentrations of *p*-nitrophenyl phosphate (Fig. 4) showed an increase in enzyme activity that reached maximum values at *p*-nitrophenyl phosphate concentrations in the range 5—10 mM and then decreased gradually. This pattern appears to be a property of this enzyme system [6,10]. Half-maximal activation by *p*-nitrophenyl phosphate (K_m) of the K^+-dependent phosphatase (as estimated from the hyperbolic region of the curve in Fig. 4) is 1.62 mM. The reason for the decrease in enzyme specific activity at high concentrations of *p*-nitrophenyl phosphate has not been elucidated. The pattern was unchanged by varying the concentration of K^+. Neither P_i nor *p*-nitrophenol, alone or combined, had any demonstrable inhibitory effects when added in vitro. This excludes the possibility of product inhibition. The pattern of activation by K^+ of the phosphatase, at a fixed concentration of *p*-nitrophenyl phosphate, is depicted in Fig. 5. As in the case of the *p*-nitrophenyl phosphate activation, maximal activities (for K^+ concentration in the range 7.25—20 mM)

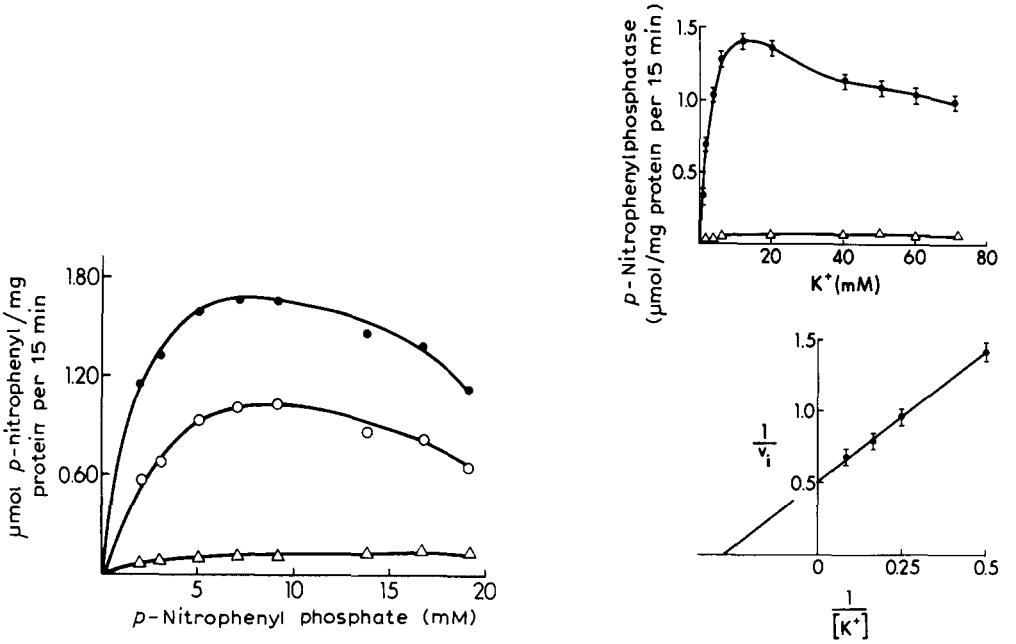


Fig. 4. Specific activity of rat renal cortical *p*-nitrophenylphosphatase as a function of the concentration of *p*-nitrophenyl phosphate. Double-reciprocal plots of the hyperbolic region of the curve (*p*-nitrophenyl phosphate concentrations less than 10 mM) yielded a straight line (cf. Fig. 11) with an apparent K_m value for *p*-nitrophenyl phosphate of 1.62 mM. ●, K^+-activated; ○, ouabain-sensitive; △, Mg^{2+}-activated.

Fig. 5. Specific activity of rat renal cortical *p*-nitrophenylphosphatase as a function of the concentration of K^+. The double-reciprocal plot (lower panel) was constructed from the hyperbolic region of the activation curve (K^+ concentrations less than 12.5 mM) and yielded an apparent K_m value for K^+ of 3.4 mM. The values plotted are the means ±S.E. of three experiments. V_i, specific activity of *p*-nitrophenylphosphatase in μmol *p*-nitrophenol/mg protein per 15 min; *p*-nitrophenyl phosphate concentration was 5 mM.

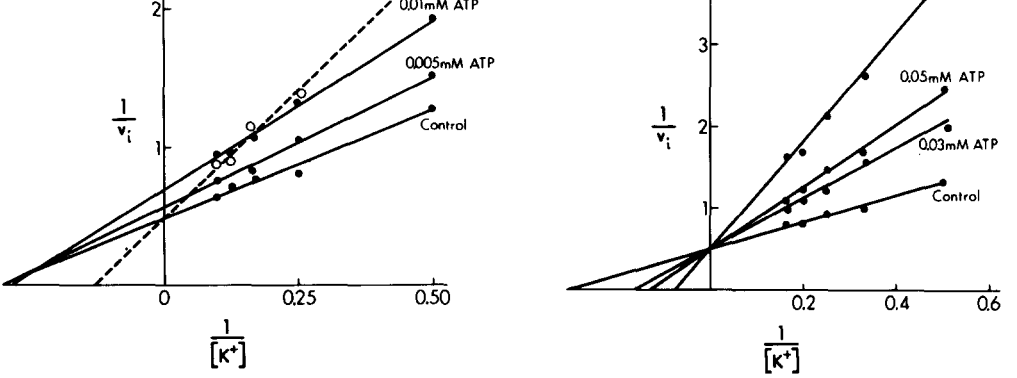


Fig. 6. Effects of low ATP concentrations (less than 0.01 mM) on K^+ activation of renal cortical *p*-nitrophenylphosphatase. A pattern of non-competitive inhibition is observed. Notice that at an ATP concentration of 0.02 mM (broken line) the inhibitory pattern appears to change (cf. Fig. 7). The *p*-nitrophenyl phosphate concentration used was 4 mM. The apparent $K_{1/2}$ value for K^+ is 3.25 mM. V_i, *p*-nitrophenylphosphatase specific activity in μmol *p*-nitrophenol/mg protein per 15 min.

Fig. 7. Effects of high ATP concentrations (greater than 0.02 mM) on K^+ activation of renal cortical *p*-nitrophenylphosphatase. A typical pattern of competitive inhibition is observed. The K_I value for ATP is approx. 0.02 mM. The $K_{1/2}$ value for K^+ in the absence of ATP is 3.05 mM. V_i, *p*-nitrophenylphosphatase specific activity in μmol *p*-nitrophenol/mg protein per 15 min; *p*-nitrophenyl phosphate concentration used was 5 mM.

are followed by a gradual decrease in enzyme activity. This pattern has been attributed to the ionic strength effect of high potassium concentrations [3,25]. The apparent $K_{1/2}$ value for K^+ activation calculated from double-reciprocal plots of the data presented in Fig. 5 is 3.4 mM. In multiple determinations with different enzyme preparations, the apparent $K_{1/2}$ value for K^+ was in the range 2.2—3.3 mM.

Effects of ATP and sodium on K^+-dependent p-nitrophenyl phosphatase. Rigorous thermodynamic analysis has led to the notion that the coupling of the free energy of ATP hydrolysis (by the $(Na^+ + K^+)$-ATPase) to transmembrane cationic fluxes (Na^+, K^+) requires that the enzyme system be anisotropic [13, 27—29]. Anisotropy of the enzymatic system is apparently fulfilled by the fact that the enzyme appears to span the whole thickness of the plasma membrane [28,29] and also by the presence of specific binding sites for Na^+ and ATP on the inside of the bilayer and of binding sites for K^+ on the outside of the bilayer [13,23,27]. If $(Na^+ + K^+)$-ATPase and *p*-nitrophenylphosphatase form part of the same enzymatic system, it may be predicted that the activity of the phosphatase may be modified by substrates that bind to the enzymatic complex on the inside or outside of the transmembrane enzymatic complex. It has been reported that ATP inhibits *p*-nitrophenylphosphatase activity by changing the affinity for both *p*-nitrophenyl phosphate and K^+ [3,9,25,26,30]. In the kidney, the inhibitory effect of ATP on the activation of the phosphatase by *p*-nitrophenyl phosphate displays a pattern consistent with competitive inhibition [19], which may indicate that the binding sites for ATP are similar to those for K^+ and *p*-nitrophenyl phosphate. However, as pointed out by Garrahan et al. [30], since hydrolysis of phosphatase substrates is probably a multistage reaction, the kinetic data do not necessarily mean that substrates and inhibitors combine at the same site. We examined in detail the interaction of ATP with respect to the activation of the phosphatase by K^+ at optimal concentrations of *p*-nitrophenyl phosphate (Figs. 6 and 7). ATP appears to have a dual effect on the K^+ activation of *p*-nitrophenylphosphatase: at low ATP concentrations (below 0.02 mM), ATP behaves as a non-competitive inhibitor of the phosphatase (Fig. 6). The apparent K_i value for this effect is 0.015 mM. At concentrations of ATP higher than 0.02 mM, a typical pattern of competitive inhibition was obtained (Fig. 7). The apparent K_i value for this effect of ATP was 0.04 mM, a value which is substantially lower than that reported for ATP inhibition of acetyl phosphate activation of the phosphatase in guinea-pig kidney (2 mM) [3], but similar to the value obtained for inhibition of the phosphatase in human kidney (0.051 mM) [19]. The pattern of ATP inhibition at low concentrations is consistent with interaction of this substance at a binding site different to the active site for K^+ and *p*-nitrophenyl phosphate; the pattern obtained at higher ATP concentrations is consistent with interaction at the same binding site. The inhibitory effect of ATP on the activation by *p*-nitrophenyl phosphate of the phosphatase was also remarkable in that at high ATP concentrations (2—3 mM), the reduction in the activity of the K^+-dependent phosphatase was accompanied by a corresponding increase in the specific activity of the Mg^{2+}-activated phosphatase (Fig. 9).

Studies with microsomal fractions from brain, red blood cells and the elec-

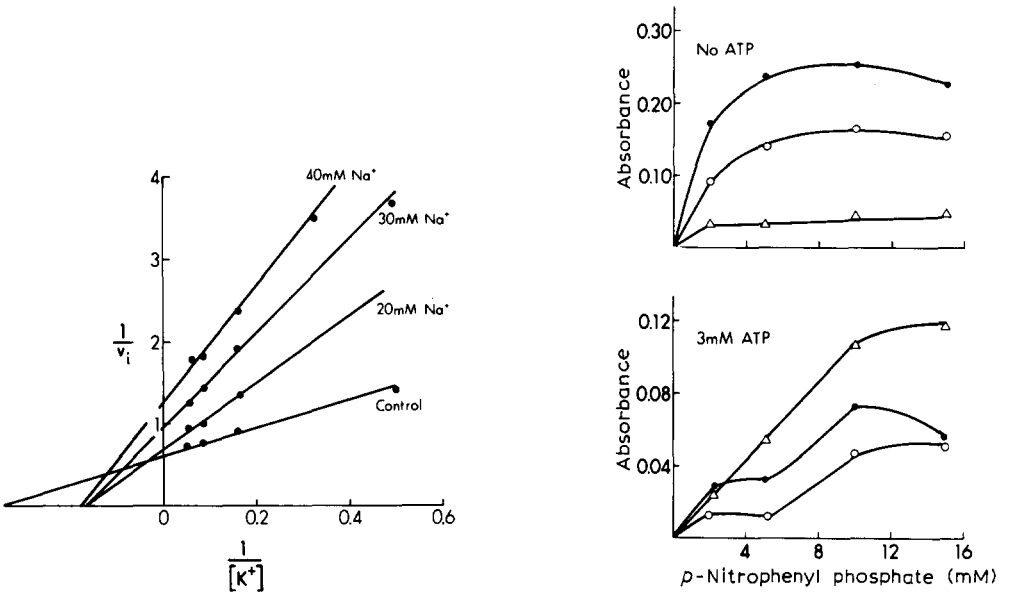


Fig. 8. Effects of Na^+ on K^+ activation of renal cortical *p*-nitrophenylphosphatase. A change in the affinity and *V* occurs with the lowest concentration of Na^+ (20 mM). Higher Na^+ concentrations yield a progressive decrease in *V* but no further change in K_m. The apparent K_m value for K^+ in the absence of Na^+ is 2.8 mM and in the presence of Na^+, 9.8 mM. V_i, *p*-nitrophenylphosphatase specific activity in μmol *p*-nitrophenol/mg protein per 15 min.

Fig. 9. Effects of ATP (3 mM) on the activation of *p*-nitrophenylphosphatase by *p*-nitrophenyl phosphate. Specific activities of K^+-activated and ouabain-sensitive *p*-nitrophenylphosphatase are markedly inhibited and that of Mg^{2+}-activated *p*-nitrophenylphosphatase is greatly enhanced. The pattern of inhibition did not follow Michaelis-Menten kinetics. K^+ concentration used was 10 mM. •, K^+-activated; ○, ouabain-sensitive; △, Mg^{2+}-activated.

tric organ of *Electrophorus* [6,26,31] have shown that Na^+ activates [31] or inhibits [6,25,26] the activity of the phosphatase. This effect appears to depend on the K^+ concentration relative to that of Na^+. We investigated the effects of Na^+ on phosphatase activity over a wide range of K^+ concentrations (Fig. 8). We did not observe stimulation by Na^+ of the phosphatase at any concentration of K^+. Instead, a consistently inhibitory effect was observed (Fig. 8). Addition of concentrations of Na^+ as low as 5 mM produced a substantial decrease in the affinity of phosphatase for K^+ (from 2.8 to 6 mM). However, higher concentrations of this cation did not further modify the affinity of the enzyme for K^+; instead, the *V* value of the reaction was progressively reduced. This pattern suggests that the inhibitory effect of Na^+ is basically of a non-competitive type, but the overall effect is a complex one as indicated by the observed change in affinity that appears to be independent of the inhibitor's concentration. This behavior, however, may be consistent with interaction of Na^+ at a site different to the K^+ binding site. Alternatively, these results may be explained by interaction of Na^+ and K^+ with binding sites regulating substrate accessibility and/or catalytic potential of the enzyme as suggested by Albers and Koval [31].

Interaction of ouabain with p-nitrophenylphosphatase

One of the crucial arguments to establish the connection of the K^+-dependent phosphatase with the (Na^+ + K^+)-ATPase has been the fact that both activities can be inhibited by cardiac glycosides [1,3,6,9,26]. Although some studies have reported that the sensitivity to ouabain of the two enzymatic activities is similar [3,32], others have shown that the K^+-dependent phosphatase is significantly less sensitive than the (Na^+ + K^+)-ATPase to the inhibitory effects of the glycosides [9]. As shown in Fig. 10, ouabain inhibited the K^+-dependent phosphatase in a dose-related manner. Maximal inhibition obtained was 80% of the basal activity and the $K_{1/2}$ value for this effect was 0.31 mM. Preincubation of the enzyme suspension with different concentrations of ouabain resulted in complete inhibition of enzyme activity at high concentrations (1 mM) of the glycoside (Fig. 10). In addition, preincubation with ouabain resulted in a small stimulatory effect of the K^+-activated phosphatase at low concentrations of the glycoside (1—100 μM) and at the same time, the $K_{1/2}$ value of the inhibitory effect was slightly increased to 0.87 mM (Fig. 10). Stimulation by ouabain of the K^+-dependent phosphatase has also been reported with enzymes obtained from brain [33]. The interaction of ouabain with *p*-nitrophenyl phosphate and K^+ is depicted in Figs. 11 and 12. The inhibitory effect of ouabain on *p*-nitrophenyl phosphate activation of the phosphatase resembles that observed with Na^+: the cardiac glycoside produced a change in the apparent K_m value for *p*-nitrophenyl phosphate from 1.62 to 2.9 mM which was independent of the doses of the inhibitor; increasing doses of the inhibitor resulted in a clear cut decrease in the V values of the reaction (Fig. 11). In contrast, inhibition by ouabain of the K^+ activation of the phosphatase displayed a typical pattern of non-competitive inhibition with a change in both the apparent affinity ($K_{1/2}$) and V of the reaction (Fig. 12). The effects of Ca^{2+} and ATP on the phosphatase were examined because of the observation that in red blood cells, calcium in

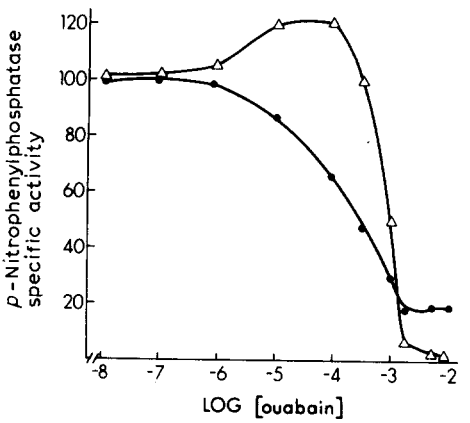
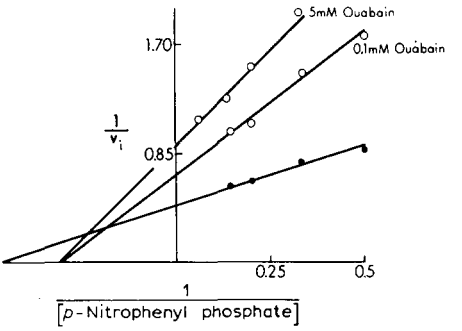


Fig. 10. Dose response of the inhibitory effect of ouabain on K^+-activated *p*-nitrophenylphosphatase. In the absence of pre-incubation (•) maximal concentrations of ouabain (greater than 1 mM) inhibited enzyme activity by 80%; the K_i value for ouabain inhibition was 0.31 mM. Pre-incubation (20 min) of the enzyme suspension with different ouabain concentrations (△) yielded a transient stimulation followed by complete inhibition (100%) of enzyme activity at ouabain concentrations greater than 1 mM; the K_i value for ouabain inhibition of enzyme subjected to preincubation was 0.87 mM.



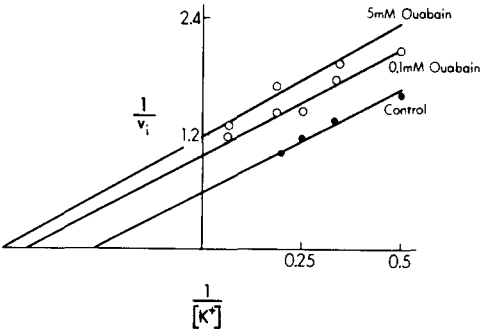


Fig. 11. Effect of ouabain on *p*-nitrophenyl phosphate of *p*-nitrophenylphosphatase. There is a decrease in the apparent affinity and V for *p*-nitrophenyl phosphate at the lower ouabain concentration (0.1 mM). An increase in ouabain concentration (to 5 mM) results in a greater decrease in V but the affinity does not change any further. The apparent K_m value for *p*-nitrophenyl phosphate in the absence of ouabain is 1.6 mM and in the presence of ouabain, 2.9 mM. K^+ concentration used was 12 mM. V_i, *p*-nitrophenylphosphatase specific activity in μmol *p*-nitrophenol/mg protein per 15 min.

Fig. 12. Effect of ouabain on K^+-activation of *p*-nitrophenylphosphatase. There is a change in both the affinity and V for K^+. The K_i value for ouabain inhibition is 0.13 mM. The p-nitrophenyl concentration used was 5 mM. V_i, *p*-nitrophenylphosphatase specific activity in μmol *p*-nitrophenol/mg protein per 15 min.

the presence of ATP results in loss of ouabain sensitivity [10]. The addition of calcium was without effect on the K^+-activated phosphatase (Table II). Low concentrations of ATP (0.01 mM, 0.1 mM) produced a dose-dependent increase in ouabain sensitivity, but the addition of Ca^{2+} was without effect on the inhibitory effect of the glycoside (Table II).

Effects of maneuvers that modify the specific activity of renal ($Na^+ + K^+$)-ATPase on K^+-dependent phosphatase

It has been reported that certain experimental maneuvers (e.g., adrenalectomy) decrease the specific activity of renal ($Na^+ + K^+$)-ATPase [34], whereas others (e.g., dexamethasone administration, potassium loading, reduction of renal mass) stimulate enzyme activity [14—16]. The effects of these experi-

TABLE II

EFFECTS OF Ca^{2+} AND ATP ON OUABAIN SENSITIVITY OF K^+-ACTIVATED *p*-NITROPHENYLPHOSPHATASE FROM RAT KIDNEY

Ouabain concentration was 0.1 mM. The values are the means ±S.E. of three experiments with cortical enzyme from different animals.

	Ouabain inhibition of K^+-activated *p*-nitrophenylphosphatase (%)
Control	50 ± 1
0.1 mM Ca^{2+}	52 ± 1
0.1 mM ATP	71 ± 1*
0.1 mM Ca^{2+} + 0.1 mM ATP	70 ± 2*
0.01 mM ATP	60 ± 2*
0.1 mM Ca^{2+} + 0.01 mM ATP	64 ± 2*

* Value statistically different from control ($P < 0.01$).

TABLE III

EFFECTS OF ADRENALECTOMY ON THE SPECIFIC ACTIVITIES OF *p*-NITROPHENYLPHOSPHATASE AND $(Na^+ + K^+)$-ATPase

Rats were adrenalectomized and maintained on 0.9% saline solution (as drinking fluid) for 5 days prior to preparations of microsomal fractions for enzyme assays. Controls were sham-operated animlas drinking saline solution. The values are the means ±S.E. The numbers of animals are shown in parentheses. n.s., not significant. Specific activities are given as μmol *p*-nitrophenol/mg protein per 15 min (*p*-nitrophenylphosphatase) and μmol P_i/mg protein per h ($(Na^+ + K^+)$-ATPase).

	p-Nitrophenylphosphatase			$(Na^+ + K^+)$-ATPase
	Mg^{2+}-activated	K^+-activated	Ouabain-sensitive	
Cortex				
Control (6)	1.35 ± 0.42	3.10 ± 0.76	1.45 ± 0.18	15.3 ± 0.7
Experimental (6)	1.26 ± 0.37	1.47 ± 0.92	0.77 ± 0.98	9.3 ± 0.4
P	n.s.	<0.01	<0.01	<0.01
Medulla				
Control (5)	1.58 ± 0.62	8.38 ± 0.96	4.15 ± 0.73	25.6 ± 1.9
Experimental (6)	1.49 ± 0.28	3.71 ± 0.31	2.31 ± 0.82	17.1 ± 1.6
P	n.s.	<0.01	<0.01	<0.01

mental maneuvers on renal *p*-nitrophenyl phosphatase were investigated and the results are presented in Tables III—VII. Because of the apparent differences in sensitivity to ouabain of *p*-nitrophenylphosphatase and $(Na^+ + K^+)$-ATPase in the kidney [9], we examined the effects of these experimental maneuvers on the K^+-activated phosphatase as well as on the fraction of this activity that is ouabain sensitive in renal cortex and renal medulla. As shown in Table III, adrenalectomy reduced the specific activity of $(Na^+ + K^+)$-ATPase in the cortex

TABLE IV

EFFECTS OF ADMINISTRATION OF MULTIPLE DOSES OF DEXAMETHASONE ON THE SPECIFIC ACTIVITIES OF *p*-NITROPHENYLPHOSPHATASE AND $(Na^+ + K^+)$-ATPase

Adrenalectomized rats received daily intraperitoneal injections of dexamethasone (1 mg/100 g body wt.) for 5 days. Controls received identical volumes of vehicle. The animals were killed 2 h after the last injection of hormone or vehicle and microsomal fractions were prepared for enzyme assays. The values are the means ±S.E. The numbers of animals are shown in parentheses. Specific activities are expressed as μmol *p*-nitrophenol/mg protein per 15 min for *p*-nitrophenylphosphatase and μmol P_i/mg protein per h for $(Na^+ + K^+)$-ATPase. n.s., not significant.

	p-Nitrophenylphosphatase			$(Na^+ + K^+)$-ATPase
	Mg^{2+}-activated	K^+-activated	Ouabain-sensitive	
Cortex				
Control (5)	1.43 ± 0.16	2.32 ± 0.25	1.33 ± 0.16	10.5 ± 1.4
Experimental (6)	1.31 ± 0.14	4.64 ± 0.39	2.87 ± 0.27	27.6 ± 1.5
P	n.s.	<0.01	<0.01	<0.01
Medulla				
Control (6)	1.03 ± 0.12	4.52 ± 0.31	2.65 ± 0.19	25.6 ± 1.5
Experimental	1.05 ± 0.10	8.47 ± 0.59	4.87 ± 0.39	55.1 ± 8.3
P	n.s.	<0.01	<0.01	<0.01

TABLE V

EFFECTS OF POTASSIUM LOADING ON THE SPECIFIC ACTIVITIES OF *p*-NITROPHENYLPHOSPHATASE AND $(Na^+ + K^+)$-ATPase

Normal rats were K^+ loaded by substituting drinking water for a 100 mM solution of KCl for 1 week prior to preparation of microsomal fractions for assay of enzyme activities. The values are the means ±S.E. The numbers of animals are shown in parentheses. Specific activities are expressed as μmol *p*-nitrophenol/mg protein per 15 min for *p*-nitrophenylphosphatase and μmol P_i/mg protein per h for $(Na^+ + K^+)$-ATPase. n.s., not significant.

	p-Nitrophenylphosphatase			$(Na^+ + K^+)$-ATPase
	Mg^{2+}-activated	K^+-activated	Ouabain-sensitive	
Cortex				
Control (5)	1.24 ± 0.10	2.19 ± 0.38	1.63 ± 0.23	19.4 ± 1.8
Experimental (6)	0.95 ± 0.07	4.31 ± 0.85	2.79 ± 0.43	35.5 ± 2.6
P	<0.01	<0.01	<0.01	<0.01
Medulla				
Control (5)	1.18 ± 0.08	5.82 ± 0.82	2.51 ± 1.2	42.3 ± 1.5
Experimental	1.05 ± 0.11	6.35 ± 1.2	2.82 ± 0.9	55.2 ± 1.4
P	n.s.	n.s.	n.s.	<0.01

and medulla of the kidney and this effect was accompanied by proportional reductions in the specific activities of both the K^+-activated and ouabain-sensitive phosphatases without a change in the specific activity of the Mg^{2+}-activated phosphatase. On the other hand, administration of multiple doses of the synthetic glucocorticoid dexamethasone to adrenalectomized rats stimulated the specific activities of $(Na^+ + K^+)$-ATPase, K^+-activated phosphatase and ouabain-sensitive phosphatase in the cortex and medulla and had no demonstrable

TABLE VI

EFFECTS OF ADMINISTRATION OF A SINGLE DOSE OF DEXAMETHASONE ON THE SPECIFIC ACTIVITIES OF *p*-NITROPHENYLPHOSPHATASE AND $(Na^+ + K^+)$-ATPase

Adrenalectomized rats were injected with a single intraperitoneal dose of dexamethasone (5 mg/100 g body wt.). Controls received an identical volume of vehicle. The animals were killed 2 h later and microsomal fractions were prepared for enzyme assays. The values are the means ±S.E. The numbers of animals are shown in parentheses. Specific activities are expressed as μmol *p*-nitrophenol/mg protein per 15 min for *p*-nitrophenylphosphatase and μmol P_i/mg protein per h for $(Na^+ + K^+)$-ATPase. n.s., not significant.

	p-Nitrophenylphosphatase			$(Na^+ + K^+)$-ATPase
	Mg^{2+}-activated	K^+-activated	Ouabain-sensitive	
Cortex				
Control (5)	1.43 ± 0.16	2.32 ± 0.25	1.33 ± 0.16	12.5 ± 1.4
Experimental (5)	1.35 ± 0.10	4.36 ± 0.11	2.43 ± 0.11	18.0 ± 0.9
P	n.s.	<0.01	<0.01	<0.05
Medulla				
Control (5)	1.03 ± 0.12	4.32 ± 0.31	2.65 ± 0.19	25.7 ± 1.4
Experimental (5)	1.09 ± 0.13	5.68 ± 0.26	3.34 ± 0.17	36.3 ± 5.5
P	n.s.	<0.02	<0.02	<0.01

effect on the Mg^{2+}-activated phosphatase. The magnitude of the stimulatory effect on $(Na^+ + K^+)$-ATPase was also comparable to that of the K^+-activated and ouabain-sensitive phosphatases (Table IV). Similar results were obtained in the cortex, in experiments with K^+ loading (Table V). In the renal medulla, activation of $(Na^+ + K^+)$-ATPase by K^+ loading was not accompanied by a corresponding increase in *p*-nitrophenylphosphatase specific activity (Table V). It should be pointed out, however, that the magnitude of stimulation of medullary enzyme by K^+ loading (approx. 30%) was substantially lower than that of cortical enzyme (approx. 83%). As shown in Table VI, administration of a single dose of dexamethasone to adrenalectomized rats stimulated $(Na^+ + K^+)$-ATPase specific activity in renal cortex and medulla. The specific activities of the K^+-activated and ouabain-sensitive phosphatases were also increased after a single dose of dexamethasone. However, the magnitude of *p*-nitrophenylphosphatase stimulation in the cortex (approx. 85%) greatly exceeded the increase in $(Na^+ + K^+)$-ATPase (approx. 44%). This discrepancy may reflect changes in the kinetic properties of *p*-nitrophenylphosphatase and $(Na^+ + K^+)$-ATPase following administration of a single dose of dexamethasone. Reduction of renal mass has been reported to increase the specific activity of $(Na^+ + K^+)$-ATPase [14]. We did not observe consistent stimulation of renal $(Na^+ + K^+)$-ATPase within 2 weeks after unilateral nephrectomy. However, in experiments that showed clear-cut stimulation of $(Na^+ + K^+)$-ATPase, there was a corresponding increase in the specific activities of the K^+-activated and ouabain-sensitive phosphatases (Table VII).

Fig 13 shows the correlation between the specific activities of $(Na^+ + K^+)$-ATPase and *p*-nitrophenylphosphatase in individual animals under all the experimental conditions examined. It is noteworthy that there was a linear correlation between $(Na^+ + K^+)$-ATPase and *p*-nitrophenylphosphatase in con-

TABLE VII

EFFECTS OF REDUCTION OF RENAL MASS ON THE SPECIFIC ACTIVITIES OF *p*-NITROPHENYLPHOSPHATASE AND $(Na^+ + K^+)$-ATPase IN NORMAL RATS

Normal rats were nephrectomized and allowed to recover for a period of 2 weeks, prior to preparation of microsomal fractions for enzyme assays. Controls were sham-operated. The values are the means ±S.E. The numbers of animals are shown in parentheses. Specific activities are expressed as μmol *p*-nitrophenol/mg protein per 15 min for *p*-nitrophenylphosphatase and μmol P_i/mg protein per h for $(Na^+ + K^+)$-ATPase. n.s., not significant.

	p-Nitrophenylphosphatase			$(Na^+ + K^+)$-ATPase
	Mg^{2+}-activated	K^+-activated	Ouabain-activated	
Cortex				
Control (5)	1.25 ± 0.12	1.35 ± 0.47	1.58 ± 0.57	13.2 ± 0.90
Experimental (6)	1.08 ± 0.10	1.65 ± 0.28	1.48 ± 0.28	14.5 ± 1.20
P	n.s.	n.s.	n.s.	n.s.
Medulla				
Control (5)	1.02 ± 0.11	2.46 ± 0.29	1.23 ± 0.29	25.6 ± 1.5
Experimental (6)	1.09 ± 0.25	4.71 ± 0.18	2.31 ± 0.18	39.6 ± 1.8
P	n.s.	<0.02	<0.02	<0.01

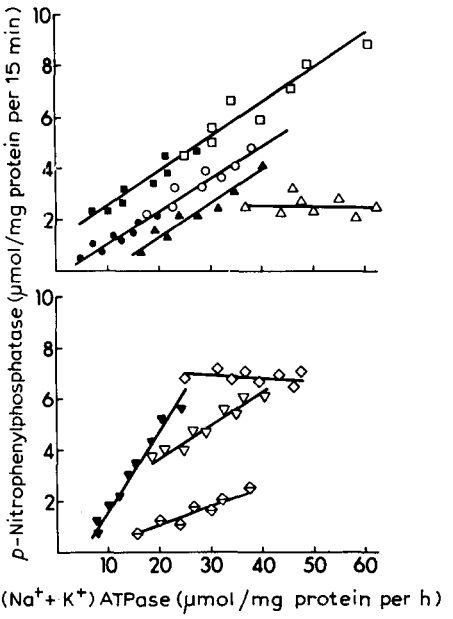


Fig. 13. Correlation between the specific activities of renal $(Na^+ + K^+)$-ATPase and *p*-nitrophenylphosphatase under different experimental conditions. Each point corresponds to an individual animal. The closed symbols are the values for enzyme obtained from renal cortex and the open symbols for enzyme from renal medulla. Control and experimental values for each condition are plotted. The upper panel depicts the results obtained with adrenalectomy (●,○), chronic dexamethasone administration (■,□) and potassium loading (▲,△). The lower panel depicts the results obtained with acute dexamethasone administration (▼,▽) and reduction of renal mass (◇,⊖). The values for cortical enzyme after reduction of renal mass have not been plotted because stimulation of $(Na^+ + K^+)$-ATPase following this maneuver did not occur in the cortex. In the medulla, some experiments showed stimulation of $(Na^+ + K^+)$-ATPase specific activity (⊖) but others failed to do so (◇) (see text). The *p*-nitrophenylphosphatase values plotted correspond to the K^+-activated fraction. For some of the experiments on reduction of renal mass (◇), the ouabain-sensitive fractions have been plotted. The slopes for the different lines are as follows: adrenalectomy (●,○): 0.124, $r = 0.94$, $P < 0.01$; chronic dexamethasone (■,□): 0.138, $r = 0.96$, $P < 0.01$; potassium loading (cortex only) (▲): 0.132, $r = 0.96$, $P < 0.01$; acute dexamethasone (cortex) (▼): 0.363, $r = 0.92$, $P < 0.02$; acute dexamethasone (medulla) (▽): 0.119, $r = 0.91$, $P < 0.02$; reduction of renal mass (⊖): 0.058, $r = 0.88$, $P < 0.05$.

trol and experimental animals under conditions of adrenalectomy, chronic dexamethasone treatment and K^+ loading. This applied to enzyme obtained from cortex and medulla, except after K^+ loading in which case stimulation of medullary $(Na^+ + K^+)$-ATPase was not accompanied by a corresponding change in *p*-nitrophenylphosphatase (Table V, Fig. 13). Moreover, the slope of the lines describing these relationships were virtually the same (0.124—0.136; $r = 0.92$, $P < 0.01$) in the three experimental situations. In the experiments on acute dexamethasone treatment and reduction of renal mass, there was also a linear correlation between $(Na^+ + K^+)$-ATPase and *p*-nitrophenylphosphatase in control and experimental animals, but the slopes of the corresponding lines were either greater (acute dexamethasone: 0.37; $r = 0.91$, $P < 0.01$) or smaller (reduction of renal mass: 0.06; $r = 0.86$, $P < 0.05$) than under the other three experimental conditions. These results are consistent with the interpretation that the changes in renal $(Na^+ + K^+)$-ATPase specific activity after adrenalec-

tomy, chronic dexamethasone treatment, K^+ loading, reduction of renal mass and acute dexamethasone treatment represent changes in the number of enzyme units. In addition, it is possible that after dexamethasone treatment and reduction of renal mass, changes in the kinetic properties of the activated enzyme may occur. To answer this question will require investigation of the kinetic properties and the rates of synthesis and/or degradation of the transport enzyme.

Discussion

The results of these experiments strengthen the inference that in the rat kidney ($Na^+ + K^+$)-ATPase and K^+-activated phosphatase are part of the same enzymatic unit. If ($Na^+ + K^+$)-ATPase and *p*-nitrophenylphosphatase correspond to the same enzymatic unit, it then follows that changes in the specific activitiy of ($Na^+ + K^+$)-ATPase should be accompanied by proportional changes in the specific activity of the phosphatase. This prediction has been tested in microsomal enzyme preparations obtained from rat heart (ventricular muscle) in which administration of thyroid hormone results in proportional stimulation of ($Na^+ + K^+$)-ATPase and *p*-nitrophenylphosphatase [35]. Our results show that this prediction is fulfilled in the rat kidney under the experimental conditions examined. However, following acute dexamethasone administration and reduction of renal mass, stimulation of ($Na^+ + K^+$)-ATPase specific activity is not accompanied by a proportional increase in the activity of the phosphatase. Although the latter results may in principle argue against the concept of the two activities corresponding to the same enzyme unit, other interpretations are possible. A given experimental maneuver that modifies the specific activity of renal ($Na^+ + K^+$)-ATPase may do so either by affecting the number of enzyme units or by modifying the kinetic properties of pre-existing enzyme units. A change in the number of enzyme units may be the result of changes in the rate of synthesis and/or degradation or it may simply reflect the unmasking of latent units. Since hydrolysis of ATP by ($Na^+ + K^+$)-ATPase appears to be a two-step reaction in which sodium stimulates the production of a high-energy phosphorylated intermediate followed by a potassium-dependent dephosphorylation [13] and the sodium and potassium regulatory sites appear to be located on different regions of the enzyme [13,23,27], it may be possible to modify the catalytic activity of the overall reaction by allosteric effects confined to the sodium- and/or potassium-dependent sites. If this were the case, and one further assumes that the phosphatase activity is a reflection of the potassium-activated site of ($Na^+ + K^+$)-ATPase, then it follows that a change in the specific activity of ($Na^+ + K^+$)-ATPase secondary to an allosteric effect on sites other than the potassium binding site might not be reflected in a proportional increase of the phosphatase activity. By the same token, any change in ($Na^+ + K^+$)-ATPase activity secondary to differences in the number of enzyme units must be reflected in a proportional change in the activity of the phosphatase. The results of experiments with adrenalectomy, chronic dexamethasone administration and potassium loading, in which the changes in the two enzyme activities were quantitatively correlated, suggests that under these experimental conditions the changes in ($Na^+ + K^+$)-ATPase specific activity

are best explained by differences in the number of enzyme units. This interpretation is strengthened by prior studies on the kinetic behavior of $(Na^+ + K^+)$-ATPase following adrenalectomy [34] in the rat. On the other hand, the apparent dissociation in the magnitude of the changes in specific activities of $(Na^+ + K^+)$-ATPase and *p*-nitrophenylphosphatase that we observed following acute dexamethasome administration and reduction of renal mass may indicate that under these experimental conditions the increase in $(Na^+ + K^+)$-ATPase specific activity cannot be accounted for solely by an increase in the number of enzyme units and that allosteric modification of pre-existing enzyme units, may also contribute to the observed increase in enzyme activity. Obviously, further experiments designed to investigate the partial reactions of the $(Na^+ + K^+)$-ATPase as well as the rate of synthesis and degradation of this enzyme will be required to define ultimately this problem. Our results, however, underscore a potential use of the phosphatase reaction in examining the mechanisms of activation and/or inhibition of $(Na^+ + K^+)$-ATPase in the kidney. Our studies also suggest that in spite of the rather marked differences in sensitivity of *p*-nitrophenylphosphatase and $(Na^+ + K^+)$-ATPase to ouabain the magnitude and direction of the changes in both the K^+-activated phosphatase and ouabain sensitive phosphatase were virtually the same under all the different experimental conditions tested, thus suggesting that the two activities belong to the same enzyme unit.

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