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THE EFFECT OF UREA, SODIUM AND CALCIUM ON MICROSOMAL ATPase ACTIVITY IN DIFFERENT PARTS OF THE KIDNEY

YEHUDA GUTMAN AND YAFFA KATZPER-SHAMIR

Department of Pharmacology, The Hebrew University–Hadassah Medical School, Jerusalem (Israel)

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SUMMARY

Mg^{2+}-ATPase and $(Na^+ + K^+)$-dependent ATPase activities were assayed in the microsomal fractions of the cortex, medulla and papilla of the guinea-pig kidney. Mg^{2+}-ATPase in any part of the kidney was unchanged in the presence of 300 mM urea. $(Na^+ + K^+)$-dependent ATPase activity was unchanged in the cortex, but was significantly inhibited in the medulla and papilla by 300 mM urea. Elevated concentrations of Na^+ (200 mM) depressed ATPase activity in the microsomal fraction derived from the papilla and medulla, but did not significantly affect the activity in the kidney cortex. Ca^{2+} (5 mM) caused a significant inhibition of $(Na^+ + K^+)$-dependent ATPase in all three parts of the kidney and depressed Mg^{2+}-ATPase activity only in the papilla.

Active Na^+ transport in the kidney has been shown to take place in different locations along the nephron: the proximal convoluted tubule[1,2], the loop of Henle[3], the thick ascending limb[4], the distal convoluted tubules[4,5] and the collecting duct[5]. It is assumed that sodium transport from the lumen of the nephron outwards subserves different functions in the various portions of the tubules. Thus, reabsorption of Na^+ in the proximal tubule is related to regulation of Na^+ balance; active transport along the loop of Henle is essential for the maintenance of the osmotic gradient in the kidney[3] and thus for the mechanism of urine concentration; active transport of Na^+ along the thick ascending limb enables the production of hypotonic fluid[4] and, therefore, is essential for urine dilution.

Although active transport of Na^+ is involved in each of these sites the effect of diuretic drugs, which inhibit Na^+ transport, is apparently restricted to specific portions of the nephron[6,7]. Furthermore, the characteristics of Na^+ transport differ along the nephron: while Na^+ absorption is isotonic in the proximal tubule[4,5], it is against a concentration gradient in the thick ascending part of the loop and in the distal tubule[1,4,5].

Many reports have pointed to the relationship between active Na^+ transport and the activity of a microsomal $(Na^+ + K^+)$-activated ATPase[8,9]. This enzymic activity has also been shown to occur in the kidney[10] and is found in all its parts: cortex, medulla and papilla[11–13]. If the same enzyme is involved in Na^+ transport at the different locations of the nephron it would be of interest to study whether the differences in the characteristics of Na^+ transport in various parts of the kidney are reflected in differences in the characteristics of microsomal ATPase from these parts.

Microsomal ATPase activity is usually assayed *in vitro* in isotonic media resembling the composition of plasma[8]. Cells located in the medulla and particularly in the papilla of the kidney are bathed by a hypertonic medium, particularly rich in urea and Na^+, in contrast to cells in the cortex which are perfused by an isotonic fluid. We have, therefore, studied the effects of increased Na^+ concentration and of elevated urea concentration on microsomal ATPase from the kidney cortex, medulla and papilla.

Guinea-pigs were killed by a blow on the head, the kidneys were immediately removed and placed in ice-cold 0.25 M sucrose containing 2 mM EDTA. The kidneys were cut sagitally and then divided into three portions: the papilla (the pale portion, projecting into the pelvis), the medulla (the dark-red portion, which contains the thick ascending part of the loop of Henle) and the cortex (the outermost part of the kidney). The different portions of the kidneys were homogenized in 10 vol. of the medium, then centrifuged at 1000 $\times$ *g* for 10 min. The supernatant was centrifuged at 10000 $\times$ *g* for 10 min; the supernatant from this centrifugation was then subjected to 105000 $\times$ *g* for 30 min to sediment the microsomes. The supernatant was discarded and the microsomes were resuspended in homogenizing medium.

Mg^{2+}-ATPase activity and $(Na^+ + K^+)$-dependent ATPase activity were distinguished by incubating in two different media. For total ATPase activity incubation was carried out in a medium containing 100 mM NaCl, 10 mM KCl, 4 mM $MgCl_2$, 4 mM ATP (sodium salt) and 33 mM Tris buffer (pH 7.2). For Mg^{2+}-ATPase activity NaCl and KCl were replaced by Tris, and the sodium salt of ATP was replaced by Tris–ATP. The difference between the total ATPase activity and the Mg^{2+}-ATPase activity was the $(Na^+ + K^+)$-dependent activity. The results thus obtained were identical with the $(Na^+ + K^+)$-dependent ATPase activity determined by incubation in the complete electrolyte medium in the presence of 1 mM ouabain.

The incubation was carried out for 20 min at 37° with continuous shaking. The reaction was stopped by the addition of 1 vol. of 5 % trichloroacetic acid. Inorganic phosphate was determined according to BAGINSKI AND ZAK[14], and protein by the method of LOWRY *et al.*[15].

To study the effect of urea, 300 mM urea (final concentration) was added either to the medium for Mg^{2+}-ATPase or to the medium containing NaCl and KCl. No significant changes in the activity of Mg^{2+}-ATPase were observed in the presence of urea in any portion of the kidney: cortex, medulla or papilla. However, $(Na^+ + K^+)$-dependent ATPase activity was affected by 300 mM urea. Both in the medulla and in the papilla a significant decrease in $(Na^+ + K^+)$-dependent ATPase activity was observed (Table I), the inhibition being 12.6 and 36.0 %, respectively. No significant inhibition of $(Na^+ + K^+)$-dependent ATPase activity was observed in the kidney cortex.

To test the effect of increased electrolyte concentration, incubation was carried out in a medium containing a final concentration of 200 mM NaCl and 20 mM KCl. (The K^+ concentration was increased in order to keep the ratio $Na^+ : K^+$ the same as in the control medium.) As seen in Table I, increased electrolyte concentration also inhibited $(Na^+ + K^+)$-dependent ATPase activity differently in the various parts of the kidney: no significant effect on cortical ATPase was noted, but there was a significant inhibition in the medulla and papilla (12.2 and 27.9 %, respectively).

Ca^{2+} has been reported to inhibit the activity of $(Na^+ + K^+)$-dependent ATPase

from kidney and other tissues[16]. However, no comparison was made between the effects of Ca^{2+} on different parts of the kidney. It has been observed that hypercalcemia specifically decreases the concentrating capacity of the kidney[17], and inhibits Na^+ reabsorption[18]. We have, therefore, studied the effect of Ca^{2+} (final concentration

TABLE I

EFFECTS OF UREA AND Na^+ ON MICROSOMAL ATPase ACTIVITY IN DIFFERENT PARTS OF THE KIDNEY

ATPase activities and the decrease in ATPase activities are given in μmoles P_i released/mg microsomal protein per h. Results are expressed as means ± S.E. n = number of experiments. P values are for the differences between the activities in the presence of high urea (or Na^+) and the corresponding control values of the same preparation. N.S. = difference not statistically significant.

	$(Na^+ + K^+)$-dependent ATPase	*Decrease of $(Na^+ + K^+)$-dependent ATPase by urea*	*Inhibition of $(Na^+ + K^+)$-dependent ATPase by urea (%)*	*Decrease of $(Na^+ + K^+)$-dependent ATPase by Na^+*	*Inhibition of $(Na^+ + K^+)$-dependent ATPase by Na^+ (%)*
Cortex	7.7 ± 0.4	0.34 ± 0.50 (N.S.) $n = 10$	4.4	0.48 ± 0.26 (N.S.) $n = 9$	6.2
Medulla	14.8 ± 0.9	1.86 ± 0.48 ($P < 0.01$) $n = 10$	12.6	1.80 ± 0.69 ($P < 0.05$) $n = 9$	12.2
Papilla	4.2 ± 0.4	1.51 ± 0.30 ($P < 0.02$) $n = 5$	36.0	1.17 ± 0.26 ($P < 0.05$) $n = 4$	27.9

TABLE II

EFFECT OF Ca^{2+} ON MICROSOMAL ATPase ACTIVITIES IN DIFFERENT PARTS OF THE KIDNEY

ATPase activities and the decrease in ATPase activities are given in μmoles P_i released/mg microsomal protein per h. Results are expressed as means ± S.E. n = number of experiments. P values are for the differences between the activities in the presence of 5 mM Ca^{2+} and the corresponding values of the same preparation in the absence of Ca^{2+}. N.S. = difference not statistically significant.

	Mg^{2+}-ATPase	*$(Na^+ + K^+)$-dependent ATPase*	*Decrease of Mg^{2+}-ATPase by Ca^{2+}*	*Inhibition of Mg^{2+}-ATPase by Ca^{2+} (%)*	*Decrease of total ATPase by Ca^{2+}*	*Inhibition of $(Na^+ + K^+)$-dependent ATPase by Ca^{2+} (%)*
Cortex	8.8 ± 0.4	7.7 ± 0.4	N.S.	—	4.20 ± 0.90 ($P < 0.05$) $n = 3$	54.5
Medulla	13.2 ± 0.9	14.8 ± 0.9	N.S.	—	6.68 ± 1.10 ($P < 0.05$) $n = 3$	45.1
Papilla	6.5 ± 0.5	4.2 ± 0.4	1.94 ± 0.55 ($P < 0.05$) $n = 5$	29.9	2.93 ± 0.70 ($P < 0.02$) $n = 5$	23.5

5 mM) on ATPase activity, both Mg^{2+}- and $(Na^+ + K^+)$-dependent, in the different parts of the kidney. Table II shows that Ca^{2+} inhibited $(Na^+ + K^+)$-dependent ATPase from cortex, medulla and papilla. However, Mg^{2+}-ATPase from the papilla was also significantly inhibited by Ca^{2+}, whereas Mg^{2+}-ATPase from cortex and medulla were unaffected.

We have previously reported that $(Na^+ + K^+)$-dependent ATPase activity in the kidney cortex was decreased by prolonged saline administration, whereas the activity of this enzyme in the medulla was unaffected, and that during starvation $(Na^+ + K^+)$-dependent ATPase activity in the cortex increased whereas in the medulla it decreased[13]. These findings suggested different regulating mechanisms for cortical and medullary ATPase. Our present report demonstrates preferential effects of urea and increased sodium concentration on ATPase activity in the papilla and medulla. Since the papilla (and medulla) are characterized by the presence in the interstitial spaces of high concentrations of urea and Na^+, our findings might be interpreted as a demonstration of a self-regulating mechanism: when the tonicity in the papilla increased, the active "pumping" of Na^+ was decreased by inhibition of the $(Na^+ + K^+)$-dependent ATPase, whereas a decrease in tonicity would relieve the inhibition and accelerate the "pump". The kidney cortex, which also shows a $(Na^+ + K^+)$-dependent ATPase activity, is not exposed, *in vivo*, to high concentrations of Na^+ or urea, and is not affected by changes in their concentrations *in vitro*. The possible significance of the effect of Ca^{2+} on Mg^{2+}-ATPase in the papilla is not clear. It may be of interest, however, that we have found a positive correlation between Mg^{2+}-ATPase activity in the "medulla" (including medulla and papilla) and the concentrating ability of several species of rodent[19].

REFERENCES

1 K. J. ULLRICH, B. SCHMIDT-NIELSEN, R. O'DELL, G. PEHLING, C. W. GOTTSCHALK, W. E. LASSITER AND M. MYLLE, *Am. J. Physiol.*, 204 (1963) 527.
2 G. WHITTEMBURY, *J. Gen. Physiol.*, 51 (1968) 303S.
3 R. L. JAMISON, C. M. BENNETT AND R. W. BERLINER, *Am. J. Physiol.*, 212 (1967) 357.
4 C. W. GOTTSCHALK AND M. MYLLE, *Am. J. Physiol.*, 196 (1959) 927.
5 G. GIEBISCH, *Bull. Swiss Acad. Med. Sci.*, 23 (1967) 338.
6 N. BANK, *Ann. Rev. Med.*, 19 (1968) 103.
7 F. G. KNOX, F. X. WRIGHT, S. S. HOWARDS AND R. W. BERLINER, *Am. J. Physiol.*, 217 (1969) 192.
8 J. C. SKOU, *Physiol. Rev.*, 45 (1965) 596.
9 I. M. GLYNN, *Brit. Med. Bull.*, 24 (1968) 165.
10 A. I. KATZ AND F. H. EPSTEIN, *New Engl. J. Med.*, 278 (1968) 253.
11 S. L. BONTING, K. A. SIMON AND N. M. HAWKINS, *Arch. Biochem. Biophys.*, 95 (1961) 416.
12 W. PAUL AND H. C. GONICK, *Proc. Soc. Exptl. Biol. Med.*, 127 (1968) 1175.
13 Y. GUTMAN AND Y. BEYTH, *Biochim. Biophys. Acta*, 193 (1969) 475.
14 E. BAGINSKI AND B. ZAK, *Clin. Chim. Acta*, 5 (1960) 834.
15 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
16 F. H. EPSTEIN AND R. WHITTAM, *Biochem. J.*, 99 (1966) 232.
17 A. MANITIUS, H. LEVITIN, D. BECK AND F. H. EPSTEIN, *J. Clin. Invest.*, 39 (1960) 693.
18 Y. GUTMAN AND C. W. GOTTSCHALK, *Israel J. Med. Sci.*, 2 (1966) 243.
19 Y. BEYTH AND Y. GUTMAN, *Israel J. Med. Sci.*, 6 (1970) 319.