PRELIMINARY NOTES

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Effect of dehydration, starvation and sodium deprivation on microsomal ATPase in the kidney*

According to the counter-current theory[1], concentration of the urine along the kidney papilla depends on a "single effect", probably that of active sodium transport from the tubular lumen outwards[2]. The active sodium transport along the medulla[3] enables the build-up of an osmotic gradient. Under conditions of water deprivation the osmotic gradient in the papilla further increases[4].

On the other hand, many data have accumulated over the last decade indicating the relation of a microsomal (Na^+-K^+)-dependent ATPase activity and the function of active sodium transport in many organs, including the kidney[5–7]. This has even led to the suggestion of the name of "transport" ATPase for this enzyme. It has also been shown that sodium deprivation can reduce the concentrating ability of the kidney[8,9]. Thus, the available information would point to a relation between sodium availability, active sodium transport and the osmotic concentrating function of the kidney medulla. It seemed of interest, therefore, to study the activity of microsomal (Na^+-K^+)-dependent ATPase both in the medulla and in the cortex of the kidney as related to water deprivation, food deprivation and sodium intake.

To this end we have studied microsomal ATPase activity in kidneys of male guinea pigs. Kidney tissue (approx. 1 g) was homogenized in 10 vol. of 0.25 M sucrose containing 2 mM EDTA (disodium salt), and then centrifuged at 1000 $\times$ g for 10 min. The supernatant was centrifuged twice at 10400 $\times$ g for 10 min, and finally the microsomes were separated from the soluble fraction by centrifugation at 105000 $\times$ g for 30 min. The microsomes were resuspended in 2 ml of homogenizing medium. Total ATPase activity was assayed by incubating 0.2 ml of the microsomal suspension at 37° for 20 min in a medium containing 100 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, 4 mM ATP and 33 mM Tris buffer (pH 7.2); the final volume was 3 ml. To determine (Na^+-K^+)-dependent ATPase, ouabain (final concentration, 0.1 mM) was added to the medium. Higher concentrations of ouabain did not produce any further inhibition of ATPase activity. The residual activity in the presence of ouabain is Mg^{2+}-ATPase (or (Na^+-K^+)-independent ATPase). The reaction was stopped by the addition of one volume of trichloroacetic acid (5 %). P_i was determined according to BAGINSKI AND ZAK[10] and protein by the method of LOWRY *et al.*[11].

The medulla was separated from the cortex after cutting the kidney sagitally. The medulla and papilla were assayed together since ATPase activity in the papilla itself was very low, in accordance with other reports[17,18], and the amount of papillary tissue was very small compared to the medulla.

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Table I shows that water deprivation for 3 days produced no significant change in the medulla in either (Na^+-K^+)-dependent ATPase or Mg^{2+}-ATPase (compare Expt. 1, Groups 1 and 2). In the cortex, however, while total microsomal ATPase remained unchanged, a significant increase in Mg^{2+}-ATPase was noted whereas (Na^+-K^+)-dependent ATPase decreased. These findings do not support a direct relationship between (Na^+-K^+)-dependent ATPase in the medulla and the urine concentrating mechanism.

TABLE I

EFFECT OF WATER DEPRIVATION, SODIUM DEPRIVATION AND STARVATION ON MICROSOMAL ATPase IN THE KIDNEY

Microsomal ATPase activities are given as μmoles P_i released/mg microsomal protein per 20 min. Results are expressed as mean $\pm$ S.E. *n*, number of animals. Group 2: water deprivation for 72 h; Groups 3 and 4: food deprivation for 5 days and drinking fluid (water and 0.9% NaCl, respectively). *P* values denote significance of difference between groups marked with the appropriate symbol.

Expt. No.	*Group No.*	*Food*	*Fluid*	*Cortex*		*Medulla*	
				Mg^{2+}-ATPase	*(Na^+-K^+)-dependent ATPase*	*Mg^{2+}-ATPase*	*(Na^+-K^+)-dependent ATPase*
1	1 ($n=12$)	+	Water	§*6.11 ± 0.17	*2.92 ± 0.25	*6.10 ± 0.24	*6.88 ± 0.62
1	2 ($n=6$)	+	—	*7.08 ± 0.28	*1.93 ± 0.28	6.35 ± 0.71	7.18 ± 0.88
2	3 ($n=8$)	−	Water	§4.80 ± 0.21	§3.54 ± 0.26	*4.96 ± 0.34	*4.50 ± 0.31
2	4 ($n=8$)	−	Saline	5.07 ± 0.54	§2.51 ± 0.19	6.41 ± 0.81	4.04 ± 0.31
				*$P < 0.01$	*$P < 0.05$	*$P < 0.02$	*$P < 0.02$
				§$P < 0.01$	§$P < 0.02$		

In another experiment guinea pigs were deprived of food and were given as drinking fluid either water or 0.9% NaCl. Table I shows the microsomal ATPase activities in the cortex and medulla (Expt. 2, Groups 3 and 4). In the medulla, no significant difference between the groups on water and on saline was found in either total microsomal ATPase or the two different ATPase activities. In the cortex, the group on water showed a significantly higher activity of (Na^+-K^+)-dependent ATPase.

Comparison of Group 3 (water *ad libitum*) and Group 1 (water and food *ad libitum*) shows the effect of starvation on kidney microsomal ATPase activity. Table I demonstrates that under starvation there was a significant decrease in total microsomal ATPase activity in the kidney medulla and that the decrease was in both Mg^{2+}-ATPase and (Na^+-K^+)-dependent ATPase. The latter, however, was more severely reduced in food-deprived rats (decrease of 34.5%) compared to the decrease in medullary Mg^{2+}-ATPase (a decrease of 18.6%). In the cortex, there was a significant decrease in Mg^{2+}-ATPase (21.3%) but (Na^+-K^+)-dependent ATPase activity increased (by 21%), as reflected in a slight and insignificant change in the total microsomal ATPase activity in the cortex.

Analysis of all the experiments shows that the most prominent effects were found in (Na^+-K^+)-dependent ATPase in the kidney cortex; a decrease during water deprivation, an increase during sodium depletion (induced by food deprivation and water *ad libitum*) and some increase during starvation. A common denominator for

these three situations could have been a change in mineralocorticoid secretion. Increased mineralocorticoid secretion has been reported in the case of sodium deprivation[12]. It is interesting that (Na$^+$-K$^+$)-dependent ATPase in the medulla was not spared under starvation (note the considerable decrease in Group 3 compared to Group 1) and only cortical (Na$^+$-K$^+$)-dependent ATPase was specifically preserved under these conditions. This may stress the importance of sodium absorption in the cortical convoluted tubules, rather than the medullary part of the nephrons, for sodium conservation. Water deprivation can produce two opposite effects on mineralocorticoid secretion: (a) increas d plasma osmolarity, accompanied by hypernatremia, may inhibit secretion of the hormone; (b) hypovolemia can increase mineralocorticoid secretion. Our results would better fit possibility (a), however, no definite explanation can be given since aldosterone levels have not been determined.

Several reports have already shown decreased activity of (Na$^+$-K$^+$)-dependent ATPase in the kidney following adrenalectomy and increased activity of this enzyme following aldosterone administration[13–16]. However, these authors have not attempted to compare cortical and medullary ATPase activities in the kidney. Our results demonstrate that sodium deprivation (compare Groups 3 and 4, Table I) affects only cortical (Na$^+$-K$^+$)-dependent ATPase. It is interesting to note that JØRGENSEN[16] has found that increased sodium intake can decrease kidney ATPase activity even in adrenalectomized rats. It is rather difficult to explain the lack of difference in (Na$^+$-K$^+$)-dependent ATPase in either cortex or medulla of the kidney in animals on a sodium-poor diet as reported by PAUL AND GONICK[17]. However, these authors have carried out the experiments in the rat in which fresh preparations of microsomes show a rather low activity of (Na$^+$-K$^+$)-dependent ATPase; furthermore, the wide range of the ATPase activity reported in this study[17] might have obscured the difference.

Table I also shows that Mg^{2+}-ATPase activity in the medulla was not significantly different from the activity in the cortex, under the same experimental conditions. On the other hand, (Na$^+$-K$^+$)-dependent ATPase activity was higher in the medulla than in the cortex, especially in Groups 1 and 2, where medullary ATPase was 2–3 times higher than cortical activity. This finding corroborates that reported by BONTING *et al.* for the cat[18] and dog[19] kidney, *i.e.* a high ratio of medullary to cortical (Na$^+$-K$^+$)-dependent ATPase while Mg^{2+}-ATPase showed similar activities in cortex and medulla.

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