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Effects of Chloride, Nitrate and Sulfate on ATPase of Renal Cortex and Medulla (40206)

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Active NaCl reabsorption by the relatively water permeable thick ascending limb of Henle is thought to provide the primary source of osmotic work for both the dilution and concentration of urine (1). Burg and Green (2) and Rocha and Kokko (3) have reported *in vitro* perfusion studies of this segment of the rabbit nephron. Both groups found an electrical potential difference which was lumen positive in the presence of sodium chloride; this potential difference was eliminated when sulfate was substituted for chloride. They concluded that the thick ascending limb primarily transports chloride by an active process. Furthermore, the positive electrical potential was independent of the presence of sodium but was inhibited by ouabain. This finding is similar to previous reports of sodium independent oubain-inhibited chloride transport in amphibian tissues (4, 5).

Ouabain has been considered to be a specific inhibitor of Na-K ATPase (6), suggesting a role for this enzyme in active chloride transport. An investigation of the effects of chloride and other anions on Na-K ATPase activity of the rabbit kidney was therefore undertaken. Since the outer renal medulla is richly composed of ascending limbs of Henle (7), the Na-K ATPase of this zone was compared with cortex, which primarily consists of proximal tubules (7).

Materials and methods. Male New Zealand white rabbits (1.5–2.0 kg) were sacrificed by cervical dislocation. The kidneys were immediately removed, placed in iced 0.25 *M* sucrose, and longitudinally divided. The inner (or white) medulla was removed and discarded. Portions of outer (or red) medulla and cortex were then carefully separated in order to obtain tissue composed solely of one zone. The Na-K ATPase enzyme was prepared by an adaptation of the method of Jørgensen and Skou (8). ATPase activity was assayed by an adaptation of the method of Post and Sen (9). The incubation medium contained the chloride, nitrate, and sulfate salts of sodium and potassium. "Mg^{2+} ATPase" was determined in 150 m*M* sodium without added potassium. In a second tube, 30 m*M* potassium was present in addition to sodium; this activity represents "total ATPase". Na-K ATPase activity represents the difference in activity between the two tubes. The incubation media also contained 4.5 m*M* Na_2ATP, 7.5 m*M* $MgCl_2$, 0.75 m*M* EDTA, and 30 m*M* imidazole, pH 7.2. $MgSO_4$ (7.5 m*M*) was used in the chloride-free solutions. Care was taken to avoid chloride contamination of reagents. The inorganic phosphate content of the 3000*g* 20 min supernatant was assayed in duplicate by the method of Sumner (10). Protein was assayed by the method of Lowry *et al.* (11). ATPase activity is expressed as μmoles Pi/mg protein/20 min. Statistical analysis was performed using the paired *t* test.

Results. Comparative studies between the effects of chloride, nitrate and sulfate on kidney ATPase activity were performed. Tables I and II show the results of experiments with homogenates and microsomes of renal cortex and medulla. As expected, a higher activity of Na-K ATPase was obtained in the microsomal fraction. In contrast to previous reports of rat kidney (12, 13) the Mg^{2+} ATPase activities of rabbit medulla were considerably greater than those of cortex in both homogenates and microsomes.

The results show that the activity of Na-K ATPase was significantly influenced by the anionic composition of the incubation media. In all cases, the Na-K ATPase activity was highest in nitrate, and lowest in sulfate. The activity in nitrate was 3%–19% higher than in chloride, depending on the enzyme preparation. In the sulfate containing media, Na-K ATPase was 5–18% less than in chloride. There was no significant differences between

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TABLE I. EFFECTS OF ANIONS ON ATPase ACTIVITIES OF HOMOGENATES FROM RENAL CORTEX AND MEDULLA.*

	Total ATPase	Mg^{2+} ATPase	Na-K ATPase
Cortex			
Cl^-	4.2 ± 0.11[a] (n = 47)	2.5 ± 0.068[a] (n = 49)	1.7 ± 0.048 (n = 47)
NO_3^-	3.8 ± 0.09[b] (n = 48)	2.0 ± 0.050[b] (n = 48)	1.8 ± 0.059[b] (n = 48)
SO_4^{-2}	4.2 ± 0.10 (n = 27)	2.9 ± 0.080[c] (n = 27)	1.3 ± 0.042[c] (n = 27)
Medulla			
Cl^-	10.6 ± 0.23[a] (n = 50)	6.9 ± 0.18[a] (n = 50)	3.7 ± 0.084 (n = 50)
NO_3^-	9.3 ± 0.22[b] (n = 51)	5.6 ± 0.17[b] (n = 50)	3.7 ± 0.095[b] (n = 50)
SO_4^{-2}	10.1 ± 0.22[c] (n = 31)	7.1 ± 0.17[c] (n = 31)	3.0 ± 0.01[c] (n = 31)

* Data are expressed as mean ± SEM of μmoles Pi/mg protein/20 min.
[a] $P < 0.001$ between chloride and nitrate.
[b] $P < 0.001$ between nitrate and sulfate.
[c] $P < 0.001$ between chloride and sulfate. Paired t test was used for statistical analysis.

TABLE II. EFFECTS OF ANIONS ON ATPase ACTIVITIES OF MICROSOMES FROM RENAL CORTEX AND MEDULLA.*

	Total ATPase	Mg^{2+} ATPase	Na-K ATPase
Cortex			
Cl^-	9.8 ± 0.26[a] (n = 50)	5.7 ± 0.16[a] (n = 50)	4.1 ± 0.14[a] (n = 50)
NO_3^-	9.0 ± 0.25[b] (n = 48)	4.3 ± 0.13[b] (n = 48)	4.7 ± 0.17[b] (n = 48)
SO_4^{-2}	10.0 ± 0.38 (n = 29)	6.5 ± 0.22[c] (n = 30)	3.5 ± 0.2[c] (n = 29)
Medulla			
Cl^-	29.0 ± 0.66[a] (n = 39)	11.0 ± 0.37[a] (n = 40)	18.0 ± 0.80[a] (n = 39)
NO_3^-	27.9 ± 0.71 (n = 70)	8.9 ± 0.36[b] (n = 40)	19.0 ± 0.88[b] (n = 40)
SO_4^{-2}	28.0 ± 0.42 (n = 30)	18.0 ± 0.4[c] (n = 30)	14.0 ± 0.3[c] (n = 30)

* Data are expressed as mean ± SEM of μmoles Pi/mg protein/20 min.
[a] $P < 0.001$ between chloride and nitrate.
[b] $P < 0.001$ between nitrate and sulfate.
[c] $P < 0.001$ between chloride and sulfate. Paired t test was used for statistical analysis.

the effects of anions on Na-K ATPase from cortex compared with medulla.

Total ATPase activity of the enzyme preparations was also influenced by the anionic environment. Total ATPase activity was always highest in sulfate (5–8% higher than in chloride) and lowest in nitrate (4–11% lower than in chloride). Similarly, sulfate was associated with a 13% greater activity of Mg^{2+} ATPase than in chloride, whereas the Mg^{2+} ATPase in nitrate was 6–21% less than in chloride.

In order to test the possibility that chloride increased the sensitivity of Na-K ATPase to ouabain, experiments utilizing microsomes prepared from both cortex and medulla were performed. Figure 1 shows that the presence of chloride, nitrate, or sulfate did not appear to alter the inhibitory action of ouabain on Na-K ATPase activity.

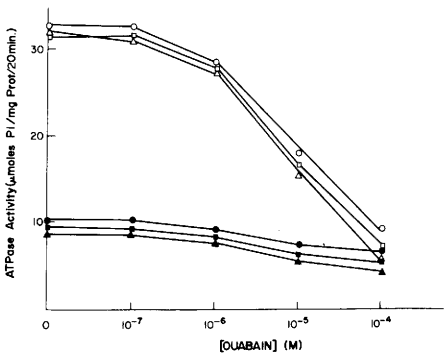

FIG. 1. Effects of anions on inhibition of Na-K-ATPase activity by ouabain. The reaction mixture contained 150 mM sodium and 30 mM potassium. Circles present studies done in sulfate, squares represent chloride, and triangles represent nitrate. Open symbols are microsomes obtained from medulla and closed symbols are microsomes from cortex.

Discussion. The purpose of the present investigation was to determine whether the inhibitory action of ouabain on active chloride transport was related to a specific interaction of this anion with Na-K ATPase. For this reason, the effect of chloride was compared with the effects of other anions on Na-K-ATPase activity of both cortex and outer medulla, the location of the thick ascending limb of Henle. Although the outer medulla

contains several portions of the nephron, the high Na-K-ATPase activity of this zone is directly attributable to the abundant thick ascending limbs (7). Rabbits were chosen because active chloride transport was demonstrated in the kidneys of this species (2, 3).

The results indicate that anions significantly affect renal Na-K ATPase activity. Nitrate was associated with a greater Na-K ATPase activity than chloride, whereas the enzyme activity was lower in the presence of the divalent anion sulfate. These differences were statistically significant but not of great magnitude, and no specific activating effect of chloride was noted. In addition, the enzyme activity of the medulla and cortex responded in qualitatively similar patterns to activation by sodium and potassium regardless of the anionic conditions. Finally, chloride did not alter the sensitivity of the enzyme to ouabain inhibition. The present results contrast with previous negative reports concerning anionic effects on Na-K ATPase. Opit *et al.* (14) and Katz and Epstein (15) found no significant effects of the anionic milieu (except for inhibition by fluoride) on Na-K ATPase activity from rat kidney, brain or heart. The large number of experiments in the present report may have facilitated detection of these effects. Alternatively, species differences may account for this disparity.

Ouabain inhibits active chloride transport by an unknown mechanism. Chloride transport may be directly or indirectly energized by ATP hydrolysis induced by Na-K ATPase, without producing an activating effect on enzyme activity. Goldin and Tong (16) reported that vessicles of purified Na-K ATPase from canine medulla transported sodium and chloride, supported by ATP hydrolysis. This transport was inhibited by ouabain. Alternately, ouabain may directly inhibit chloride transport by a mechanism independent of its action on Na-K ATPase, similar to the actions of other glycosides such as phlorizin on glucose transport (17) and atractyloside on mitochondrial adenine nucleotide transport (18).

Summary. The ascending limb of Henle actively reabsorbs chloride by a ouabain-inhibitable process. Since ouabain is considered to be a specific inhibitor of Na-K ATPase, a role for this enzyme in active chloride transport is suggested. The present investigation compares the effects of chloride, sulfate and nitrate on Mg^{2+} ATPase and Na-K ATPase of rabbit renal cortex and outer medulla. Renal Na-K ATPase activity is influenced by the anionic milieu, but the changes are not of great magnitude. There is no qualitative difference between the anion effects on the Na-K ATPase activity of cortex or outer medulla. It is concluded that chloride has no specific activating action on renal Na-K ATPase.

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1. Marsh, D. J., *in* "Osmotic concentration and dilution of the urine in the kidney" (C. Rouiller and A. F. Muller, eds.), Vol 3, p. 71. Academic Press, New York (1971).
2. Burg, M. B., and Green, N., Amer. J. Physiol. **224,** 659 (1973).
3. Rocha, A. S., and Kokko, J. P., J. Clin. Invest. **52,** 612 (1973).
4. Copperstein, I. L., J. Gen. Physiol. **42,** 1233 (1959).
5. Finn, Al L., Handler, J. S., and Orloff, J., Amer. J. Physiol. **213,** 179 (1967).
6. Glynn, I. M., Pharmacol. Rev. **16,** 381 (1964).
7. Kriz, W., Z. Zellforsch. Mikrosk. Anat. **82,** 495 (1967).
8. Jørgensen, P. L., and Skou, J. C., Biochem. Biophys. Res Commun. **37,** 39–46 (1969).
9. Post, R. L., and Sen, A. K., *in* "Methods in Enzymology" (R. W. Estabrook and M. E. Pullman, eds.), Vol. X pp. 763 Academic Press, New York (1967).
10. Sumner, J. B., Science **100,** 413 (1944).
11. Lowry, O. H., Rosebrough, N. I., Farr, A. L. and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
12. Hendler, E. D., Torretti, J. and Epstein, F. H., J. Clin. Invest. **50,** 1329 (1971).
13. Weiner, M. W., Torretti, J., Sauer, L. A., and Epstein, F. H., Amer. J. Physiol. **221,** 613 (1971).
14. Opit, L. J., Potter, H., and Charnock, J. S., Biochim. Biophys. Acta **120,** 159 (1966).
15. Katz, A. I., and Epstein, F. H., Enzyme **12,** 499 (1971).
16. Goldin, S. M., and Tong, S. W., J. Biol. Chem. **249,** 5907 (1974).
17. Wesson, L. G., *in* "Physiology of the Human Kidney" pp. 235–237, Grune and Stratton, New York (1969).
18. Vignais, P. V., Duee, E. D., Vignais, P., and Huet, J., Biochim. Biophys. Acta **118,** 465 (1966).
19. Weiner, M. W., Clin. Res. **21,** 896 (Abstract) (1973).

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