Effect of Diuretics on Sodium and Potassium Transport in the Rat's Ileum[1] (36148)

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The absorption of ions in the small intestine is influenced not only by such physiological parameters as the volume of extracellular fluid (1) and mineralocorticoid levels (2), but also by many drugs. *In vivo* studies have shown that a carbonic anhydrase inhibitor (acetazolamide) depressed sodium (Na), chloride (Cl) and H_2O absorption in the ileum of the rat (3). In addition, hydrochlorothiazide and an organomercurial diuretic (mersalyl) have been reported to inhibit the *in vitro* absorption of Na, Cl and H_2O in the rat's jejunum (4), while chlorothiazide also inhibited the *in vivo* absorption of Na in the rat's intestine (5). The effects of ethacrynic acid and furosemide on the net absorption of Na and potassium (K) in the intestine of the rat have not been investigated. The present investigation was designed to determine the effects of various diuretics on Na and K transport in an *in vitro* intestinal preparation.

Materials and Methods. Nonfasted male Holtzman rats, weighing 280–300 g, were anesthetized with ether and everted sacs of the distal ileum were prepared and mounted on a lucite cannula according to a procedure previously described (6). This procedure permitted the segments to be used for more than one incubation period. The sacs were placed in tubes containing 50 ml of Krebs bicarbonate or Krebs phosphate solutions (mucosal solution). One milliliter of the same Krebs solution containing 175 mg% inulin was placed in the everted sac through the cannula (serosal solution). All solutions contained 10 m*M* D-glucose and the pH was maintained at 7.4. Krebs bicarbonate solutions were aerated with 95% O_2 and 5% CO_2 while the Krebs phosphate solutions were aerated with 100% O_2. The preparations were incubated at 37°. Sequential one hour periods were used, and both mucosal and serosal solutions were changed at the beginning and end of each period. Serosal samples (100 μl) were taken at the beginning and end of each hour for the determinations of Na, K and inulin. The remaining volume was used for osmolality determinations. Since this preparation required 1 hr of incubation for Na and K movement to reach a steady state, the data were collected only during the first and second hour after this preincubation. The diuretics were added to the solutions at the beginning of the second hour following preincubation in the concentrations listed in Table I. Parenteral preparations of chlorothiazide (Diuril Lyovac), mercaptomerin (Thiomerin), acetazolamide (Diamox), furosemide (Lasix), and ethacrynic acid were used.

Na and K were determined by flame photometry (IL Model 143). Inulin was measured by a method which was not affected by the presence of glucose (7). Osmolality was estimated by freezing point depression with a Precision Instrument Osmette. Inulin, which is neither absorbed nor metabolized by the rat's ileum, was used to measure the net transport of water. The use of inulin as a marker in this method has been reported by others (6). Calculations are as follows:

$$\text{Fluid Movement: } Q_{H_2O} = \frac{I_i}{I_f} - 1 = \text{ml fluid transported/hr/sac}$$

Ion Movement:

$$Q_{Na} \text{ or } Q_K = (Q_{H_2O} + 1)(Na_f \text{ or } K_f) - (1 \text{ ml})(Na_i \text{ or } K_i)$$

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$= \mu$Eq of Na or K transported/hr/sac

I_i = initial (zero time) inulin conc (mg%) in 1 ml of serosal solution placed in sac; I_f = final (60 min) inulin conc (mg%) in volume of serosal solution after 1 hr; Na_f or K_f = μEq of Na or K/ml of serosal solution after 60 min; Na_i or K_i = μEq of Na or K/ml of zero time serosal solution.

All values are expressed as the mean ± standard error and statistical significance was determined by the Student *t* test.

Results. The transport of Na, K and fluid was determined in a control group of 18 everted sacs over a 2 hr period when no diuretics were added (Table I). The 2% difference in net Na transport (Q_{Na}) between the first and second hour was not significant ($p > .90$), however the Q_{Na} during the preincubation hour was 30% less than the subsequent 2 hours ($p < .01$). Therefore, the preincubation hour was not used in this study. In general, Na concentration in the serosal solution increased during the course of a 1 hr incubation. The final serosal concentration of Na was approximately 7.5 mEq/liter greater than the final mucosal concentration, and this difference will hereafter be referred to as D_{Na}. This difference in concentrations is significant in the control sacs ($p < .001$). Changes in Na and K concentration did not occur in the mucosal solution, presumably since the volume was relatively large. In the control group net K transport (Q_K) was slightly, though not significantly ($p > .30$) greater in hour 3 than in hour 2. A K concentration difference (D_K), which developed between the final serosal and mucosal solutions during 1 hr of incubation, resulted in a lower serosal concentration (negative D_K) at the end of the period. In the control group it was significantly depressed in both hours ($p < .001$). The small difference in fluid transport (Q_{H_2O}) between the 2 periods of the control group was not significant ($p > .60$). Since the Q_{Na} of hour 2 and hour 3 were not significantly different in this preparation, drugs were added at the beginning of hour 2 in the diuretic series and the ion transport data from hour 1 were used as control values. The initial osmolality of both mucosal and serosal solutions in the control group was 288.0 ± .2 mOsm. At the end of hour 1 the osmolality of serosal solution was 302.6 ± 1.2 and at the end of hour 2 it was 303.7 ± 1.2 mOsm. These differ-

TABLE I. Effects of Diuretics on Na (Q_{Na}), K (Q_K), and Fluid (Q_{H_2O}) Movement in the *In Vitro* Rat's Ileum.

Groups	No. of Rats	Hr	Q_{H_2O} ml/hr	Q_{Na} μEq/hr	Q_K μEq/hr	D_{Na} mEq/Liter/hr	D_K mEq/Liter/hr
Control	18	1	.54 ± .04[a]	89.2 ± 6.0	1.72 ± .22	8.5 ± 1.1	−.7 ± .2
		2	.57 ± .03	90.8 ± 5.8	2.07 ± .30	6.5 ± .9	−.5 ± .2
Acetazolamide	10	1	.62 ± .05	102.3 ± 9.4	2.26 ± .41	9.1 ± 1.0	−.9 ± .2
10 mg%		2	.42 ± .03[b]	66.6 ± 4.9[b]	1.46 ± .20[b]	5.3 ± 1.2	−.6 ± .1
Chlorothiazide	10	1	.70 ± .07	115.8 ± 10.4	2.19 ± .49	9.7 ± 1.0	−1.0 ± .3
8 mg%		2	.48 ± .07[b]	78.0 ± 5.6[b]	2.15 ± .44	6.3 ± .8	−.4 ± .2
Ethacrynic Acid	10	1	.55 ± .04	89.2 ± 6.8	1.67 ± .35	8.0 ± .9	−.9 ± .2
5.0 mg%		2	.57 ± .05	91.7 ± 7.9	2.37 ± .16	7.6 ± 1.2	−.5 ± .1
Furosemide	8	1	.61 ± .07	100.5 ± 11.5	1.58 ± .18	8.5 ± .6	−1.0 ± .3
1.25 mg%		2	.49 ± .05[b]	79.1 ± 8.4[b]	2.02 ± .21	5.3 ± .6	−.4 ± .2
Mercaptomerin	10	1	.47 ± .05	74.7 ± 6.8	1.25 ± .27	5.8 ± .9	−.8 ± .2
6.24 mg% (KB)		2	.47 ± .04	74.0 ± 6.3	2.24 ± .33[b]	6.3 ± 1.0	−.1 ± .3
Mercaptomerin	8	1	.50 ± .04	91.9 ± 7.6	1.32 ± .20	13.8 ± .8	−1.0 ± .1
3.12 mg% (KB)		2	.55 ± .06	99.3 ± 10.3	2.18 ± .24[b]	13.3 ± .7	−.7 ± .2
Mercaptomerin	8	1	.54 ± .06	92.9 ± 9.0	1.66 ± .30	9.8 ± 1.6	−1.3 ± .4
6.24 mg% (KP)		2	.52 ± .04	87.9 ± 6.2	2.18 ± .23	9.1 ± .6	−.5 ± .1

[a] mean ± SE.

[b] $P < .05$ (hour 2 vs. hour 1).

ences between initial and final solutions were significant ($p < .001$).

Acetazolamide inhibited Q_{Na} by 33% ($p < .001$) and Q_K by 35% ($p < .05$). In contrast the Q_K in hour 2 was always greater than hour 1 in the control group. Although chlorothiazide inhibited Na transport by 33% ($p < .001$), the Q_K was essentially the same in both hour 1 and hour 2. Ethacrynic acid did not affect Na transport in this preparation, but did cause a K movement which was greater in hour 2 than hour 1 ($p < .02$). These results were similar to the Q_K observed in the control group although in the latter the differences were not significant. Furosemide although inhibiting the Q_{Na} by 20% ($p < .001$), caused an alteration in Q_K similar to that observed in control animals. Both drug levels of mercaptomerin were added to preparations in Krebs bicarbonate (KB) solution and the high drug level was added to a group in Krebs phosphate (KP) solution. No significant changes were noted in Q_{Na} with the addition of mercaptomerin in all 3 groups. The Q_K was significantly greater in hour 2 than hour 1 in 2 of these groups. In all 7 experimental groups significant ($p < .001$) Na and K differences (D_{Na} and D_K) were observed.

Discussion. The data presented herein demonstrate that both control and experimental measurements of Na and K transport can be made on the same preparation of everted rat's ileum. This is desirable from a technical point of view, since the number of animals required to demonstrate a particular phenomenon can be substantially reduced. It is important to note, however, that a steady state in Q_{Na} is not achieved before 1 hr of preincubation. Then in the next 2 hours Q_{Na} is the same, so the first hour can serve as the control for the following experimental hour. The preincubation hour cannot be employed since Q_{Na} is significantly less than that observed in the succeeding 2 hr. A possible explanation for this phenomenon may be that the accumulation of glucose in the tissue takes approximately an hour to reach a steady state in this preparation following removal of the ileum from the animal (8). Therefore, if experiments were performed only during the first hour of incubation, the results would undoubtedly be more erratic due to the existence of nonequilibrium conditions. In addition to transporting ions equally as well in the second and third hours this preparation maintains its function to do osmotic work in both hours.

The concentrations of the diuretics in the bathing solutions were set at twice the *in vivo* levels calculated to exist following the administration of pharmacological doses as reported in the literature, and assuming that these drugs are distributed only in the extracellular fluid. Acetazolamide inhibited the Q_{Na} and decreased the Q_K. The action of this drug on Q_{Na} in the ileum may be similar to its action on carbonic anhydrase in inhibiting the exchange of Na for hydrogen ions in the kidney. In the distal tubule of the kidney, K ions compete with hydrogen ions in the exchange for Na. Therefore, if the formation of hydrogen ions is inhibited by a carbonic anhydrase inhibitor, only K will be available to exchange for Na. This would result in an increased movement of K to the mucosal side, and this is reflected in the decreased Q_K. Although *in vivo* the net movement of bicarbonate to the mucosal side of the ileum is reversed by carbonic anhydrase inhibitors (3), it has been suggested that carbonic anhydrase inhibitors do not inhibit Na transport in the intestine by inhibition of carbonic anhydrase activity (3, 9). This conclusion was based primarily on a report which denied the presence of carbonic anhydrase in the rat's intestine (10); however, utilizing more refined techniques this enzyme has recently been found in the rat's intestine (11). Thus these data suggest that the action of acetazolamide on Q_{Na} in the rat's ileum is the result of its inhibitory effect on carbonic anhydrase activity.

The inhibition of Q_{Na} by chlorothiazide was comparable to that observed for acetazolamide, but unlike the latter drug it had no pronounced effect on Q_K. Thiazide diuretics, while possessing carbonic anhydrase inhibitory activity, are not considered to act by this means in the kidney, since they do not produce the increased bicarbonate excretion seen with other more potent inhibitors of

carbonic anhydrase (12). Thus, this drug does not decrease the availability of hydrogen ions and the latter would be available to compete with K in the exchange for Na. This could explain why Q_K was not affected by chlorothiazide. An ethacrynic acid level which has been shown to produced natriuresis in rats (13) had no significant effect on Q_{Na} in the ileum of the rat. This drug is thought to inhibit Na transport in the kidney by its inhibitory effect on Na–K ATPase (14) while others suggest that this is not its mechanism of action (16). However, in the rat's ileum this ATPase is not considered to be involved in supplying energy for Na transport (15). Furosemide, at a level which produces natriuresis, inhibits Na–K ATPase (16). In the rat's ileum this diuretic depressed Q_{Na} but modestly compared to acetazolamide and chlorothiazide. This effect could be mediated by an inhibition of Na–K ATPase but the evidence obtained from ethacrynic acid in this study and from cardiac glycosides in another (15) suggests that Na–K ATPase is not involved in Na transport in the rat's ileum. The organomercurial diuretic (mercaptomerin), which is reported to inhibit renal Na–K ATPase (12), did not inhibit Q_{Na} in the ileum. This finding is not in accord with an earlier report of Rummel and Stupp who reported inhibition of sodium transport by mersalyl (4). This discrepancy may arise from the two investigations performing the experiments on different regions of the intestine.

The ileum was able to move Na and develop a concentration difference which none of these diuretics abolished. This suggests that a mechanism is present in the ileum of the rat to transport Na which is neither dependent upon carbonic anhydrase activity nor Na–K ATPase activity. In addition, this study demonstrates the requirement of carbonic anhydrase activity for another increment of Na transport by the ileal epithelial cell which may be a process of exchanging Na for hydrogen and/or K ions. Therefore, it is suggested that two or more mechanisms are present *in vitro* for the net transport of Na in the ileum of the rat.

Summary. The results of this *in vitro* study indicate that net Na transport in the ileum of the rat is not inhibited by doses of ethacrynic acid and an organomercurial diuretic which are double those pharmacologically employed to alter renal function; however, net Na transport is inhibited 35% by acetazolamide and chlorothiazide, and 20% by furosemide. These data suggest that Na transport in the rat's ileum involves at least two separate mechanisms.

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